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REVIEW ARTICLE

Rapeseed Meal as A Sustainable Plant Protein Source for Poultry in Pakistan

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ABSTRACT

The increase in the consumption of animal foods across the global population provokes worry about the resource's sustainability and the impacts on the environment before supporting plant proteins. This literature survey focuses on rapeseed meal consumption as a poultry feed substitute in Pakistan and its viability. Likewise, various methods of deactivation of anti-nutritional factors in rapeseed meals through physical, chemical, enzymatic, and plant breeding techniques are also discussed in a separate section. Therefore, the processing procedures such as acid and alkaline treatments, and extraction using solvents are described due to their efficiency in eliminating toxicants and enhancing the nutritional value of the rapeseed meal. For that reason, enzyme complexes exhibited significant functions in enhancing energy realization and nutrient assimilation within low-energy diets, which makes them significant for poultry feeds. The study makes it very important to appreciate the functional properties of canola proteins like gelation properties and solubility for several uses in the food industry. The digestibility experiment highlights how enzymes are used to enhance the level of nutrient breakdowns and absorption in poultry feeds. The review focuses on the multifaceted role of rapeseed meals as not only the source of proteins but also as a positive factor in feed production and feed recycling. To this end, the potential use of rapeseed meal in various aspects of poultry production for the kinder means of agricultural practices, environmentally friendly practices, and cost-effective practices within the poultry sector in Pakistan was determined.

1. Introduction

The task of feeding the ever-growing world population with affordable, healthy, and sustainable food remains a daunting question for humans to date [1]. A global production and consumption analysis done by the Food and Agriculture Organization (FAO) highlighted that the animal protein demand is projected to double by 2050. This is set to push the food production industry forward [1]. People's meat supply is expected to increase by more than 13% by 2026 compared to 2014-2016 and 19% compared to 2006-2008 [2]. Besides the population increase, there are issues relating to the degradation of the food production environment; more importantly, the ill-effects on groundwater, soil, and tropical forests from the interference of nitrogen, carbon, and phosphorus cycles are detrimental to meat production [3,4]. It is evident that this process also requires making the production of food more sustainable; here it is worthwhile to solve the problem of obtaining proteins from plants rather than animal-derived products to protect the environment more effectively on the whole [3]. Introducing plant-based proteins into diets is sustainable for the environment and healthy for people; it will also help with weight loss and various chronic diet-related diseases [5]. Other food trends include consuming protein from other sources or flexitarian diets; the plant-derived food market is known to be growing rapidly [1,6-7].

The mustard seeds, scientifically known as *Brassica napus*, commonly known as the oilseed rape, rapeseeds, or canola, are grown in most global regions [8]. They are made up of three different plants: Some of the popular plant varieties are black mustard, brown Indian, and white mustard. At present, this mustard seed is ranked the third-most important vegetable oil in the world after soybean oil and palm oil [9-10]. It is also second to soybean meal in terms of protein. Rapeseed production increased from 37.33 million metric tonnes in 2000 to 59.38 million tons globally [8-9]. Some of the major exporting countries are China, India, Europe, and Canada [8]. Rapeseed export trade every year is estimated to be 12 million tons on average. The UK, being a temperate region, has only one crop that is commercially viable for edible oil production and this is the oilseed rape. After oil extraction, the rapeseed meal utilized for feed is a popular high-protein meal [9]. Whole oilseed rape incorporation in feeding live stocks has also been practiced recently since the oil serves as a concentrated energy source in the diets fed to animals [10]. After soybean meal, rapeseed meal is the second most important oilseed meal produced globally. Its use has increased significantly in the USA, China, and the EU, where there is a shortage of protein feed, as well as in the EU because of the need for feed from milk producers. Canada and India are the two countries that export the most rapeseed meals worldwide. [9].

Rapeseed is the second most produced oilseed worldwide and it is identified to contain better quality protein due to better nutritional profile, balanced amino acid composition and also having better functional characteristics. It is useful as an alternative in the plant-based market with the market leader being soy protein. Besides the high cultivation volume, rapeseed is known it be resistant to different types of soil [11-14]. Rapeseed, an annual, herbaceous crop included in the family of Brassicaceae, is mostly cultivated for seeds that have 40-50% oil for human consumption [15]. Canola was developed by using rapeseed and classical breeding techniques with a focus on enhancing oil quality through glucosinolates as well as erucic acid reduction. Commonly, the glucosinolates concentration in the meal residue is < 30 µmol/g or even less, and the erucic acid level in oil is 2% or less. 'Canola' is defined as either *Brassica napus* or *Brassica rapa* improved germplasm, while 'canola-quality juncea' is defined as improved juncea germplasm. Nevertheless, among these Brassica species, *B. napus* canola is the most cultivated across the globe, and, more than 95% of the seeds generated in Canada belong to this species. Canola is familiar more in Canada and Australia while in Europe the term rapeseed or double zero rapeseed which represents low glucosinolate and erucic acid is widely used [13, 16-17].

This review focuses on analysing the real possibility of including rapeseed meals in the poultry diets in Pakistan as it is a sustainable plant-based protein resource compared with soybean meal. The specific objectives of the study will include establishing the nutritional quality, the potential methods of converting rapeseed meal to poultry feed, the impact the incorporation of rapeseed meal to poultry feed could bring to the environment, and the economics of using rapeseed meal on poultry feed. Thus, by investigating the possibility of substituting rapeseed meal as a sustainable protein source in poultry diets, the study aims to create awareness in the right direction in the context of sustainable agriculture that has minimal damaging effects on the environment while improving the economic efficiency of poultry production in Pakistan.

1.1 Literature Retrieval

Following the PRISMA guidelines, a systematic literature retrieval process was undertaken to identify related knowledge on rapeseed meal usage in poultry nutrition, focusing on anti-nutritional factors, enzyme complexes, and sustainability. A comprehensive search was carried out across databases such as PubMed, Scopus, Web of Science, Science Direct, IEEE Xplore, Elsevier, Springer Link, Wiley Online Library, Taylor & Francis Online, Sage Journals, ACM Digital Library and DOAJ (Directory of Open Access Journals) using keywords like Rapeseed meal, Poultry Nutrition, Anti-nutritional factors, Enzyme complexes, Sustainability, Processing techniques, Feed efficiency, Plant-based proteins, Protein digestion, Nutrient assimilation, Feed production, and Feed additives and a total of 300 peer-reviewed review papers, research papers, and chapters were downloaded. Additional sources including Google Scholar and institutional repositories were also consulted. Screening of titles and abstracts, followed by full-text assessment, was performed to select studies meeting the eligibility criteria. Data extraction involved capturing key information on study design, findings, and implications for poultry nutrition. Quality assessment was conducted to evaluate the relevance and methodology of the selected studies. Data synthesis was carried out to provide a cohesive overview of the literature, emphasizing the impact of rapeseed meal on poultry nutrition and sustainability.

Out of 250, 200 were shortlisted based on titles and abstracts, and relevant 169 articles were selected for this chapter. This chapter is divided into a total of eight sections. Section 1 is about the introduction, section 2 is about an overview of the poultry industry in Pakistan, and section 3 is related to identifying sustainable sources of protein for poultry as well as it comprises a brief overview of plant protein sources in Pakistan such as grain-by-products, grain legume or pulses, oilseed or fruit by-products, moreover, limitations in using these alternative protein sources was highlighted. Section 4 includes an evaluation of rapeseed meal as a promising alternative (its replacement value to other protein sources as well as its cultivation and processing in Pakistan and section 5 provides detailed knowledge about nutritional composition (feeding values of rapeseed meal, digestibility, allergenicity, the functionality of proteins such as solubility, emulsifying properties, foaming properties, and gelation). Moreover, section 6 includes a detailed description of poultry responses to diets containing sustainable protein sources: rapeseed and section 7 is about improving the nutritive value of sustainable protein sources for poultry (crop breeding, processing, nutrient supplementation, and non-nutrient supplementation). Section 7 includes the impact of anti-nutritional factors on production and productivity and treatment methods and the review ends with concluding remarks and recommendations for future studies.

2. Overview of the Poultry Industry in Pakistan (2022-2023)

Commercial Poultry in Pakistan has emerged as one of the largest segments of agro-based industry in the country with investment crossing over 1981.63 billion rupees. Its major achievement is in providing meat protein for almost every meal for most people in the world while the red meat protein source seems to be on the decline. Poultry is acknowledged, perhaps, as the cheapest source of animal protein by the populace thus helping check any hikes in prices of animal protein and providing employment to more than one. action can influence about 5 million people directly and indirectly [18]. The growth rate of the Pakistan Poultry Industry is estimated to be a turnover of 63 billion rupees besides the fact that it gives employment and income to over 5 million people. For the record, poultry is the most developed subsector of the agro-based division in the overall economy of Pakistan which has witnessed an average annual growth of 10-12%. Agriculture produces and by-products where more than 15000 poultry farms across Pakistan from Karachi to Peshawar are found and have the capacity of raising about 5000 to 500000 broilers. However, the marketing channels of both broiler and eggs predominantly reside in the unorganized segment [18]. Poultry products are a major contributor to meat consumption. Each year, the sector generates around 1153 million kilograms of chicken meat and 10,000 million table eggs. Despite considerable output, Pakistan consumes less meat than the WHO recommends, with per capita consumption standing at 5.24 kg and 45.4 eggs a year, respectively. Poultry feed is a major contributor to output, with an estimated 5.91 million metric tonnes produced each year. The poultry business's broiler and layer parts contribute significantly to total income, and the industry has a high turnover rate. Another important part of the sector is vaccines, where billions of rupees are spent every year to prevent sickness [18]. In brief, Pakistan's commercial poultry sector is rather large, producing moderately cost-effective animal protein and employment. With a turnover of 63 billion rupees, it represents 40-45% of the world's meat consumption.

3. Identifying Sustainable Sources of Protein for Poultry

Approximately 60-80% of the overall production costs in intensive chicken farming are made up of feed, of which protein makes up a large share. However, the costs of feedstuffs have increased significantly due to the growing demand for chicken feeds and the expanding poultry sector. Traditional chicken protein sources are expected to become costlier and infrequent soon [19]. Moreover, when the feedstuffs cost rises, conventional protein sources like fishmeal and oil seed meals (SBM and canola meal) are finding it harder to provide the growing need brought on by population and industry expansion. These changes make it more and more important to think about using alternative protein supplements in chicken feed to either partly or completely replace traditional protein sources. If a product is affordable, easily accessible, and has a balanced nutritional profile, it might be considered a good alternative protein replacement. But when adding different protein sources to poultry diets, one must take technical and nutritional aspects into account [20]. Some of these aspects comprise nutrient variability, the balance of necessary amino acids, toxic or antinutritional substances presence like tannins and enzyme inhibitors, pathogenic bacteria, as well as processing needs. Seasonality, texture, requirements for processing, and long-term supply are among the technical factors to be taken into account. Whether these were derived from plants or animals, unconventional protein sources often require thorough processing before being included in meals [19]. In brief, restricted and more expensive traditional protein sources are emerging due to the growing chicken business and rising consumer demand. Other protein supplements must be used in place of more conventional ones, with consideration for both nutritional and technical factors.

3.1 Grain By-Products as Alternative Plant Protein Sources in Pakistan

Grain cereals are used for both human and industrial uses, as well as animal feed. Brewers' grain (BG) and distiller’s dried grains (DDGS) with solubles are important byproducts obtained from the fermentation and distillation of starchy grains used to make ethanol and drinks. BGs from beer and malt production provide fibre and protein for animal feed [21]. Because of starch-free after conversion, DDGS (a byproduct of ethanol production) have higher levels of protein, fibre, fat, and minerals, yet are still nutrient-rich. Low lysine digestibility is associated with excessive phosphorus bioavailability [22]. The high crude fibre content of DDGS and BG, primarily non-starch polysaccharides, limits their use in chicken feed. The nutrient content of DDGS varies greatly, with low ether extract levels seen in certain samples [23-24]. Gluten feed and meal are byproducts of starch processing. Their sources are sorghum and maize, which are well-known for their high protein content []. Compared with soybean meal, maize gluten meal has more crude protein level (around 65%) but less lysine. Corn bran and steep liquor make up corn gluten feed, which varies in composition depending on the milling techniques used. At least 26.0% protein is present in sorghum gluten feed (SGF), but sorghum gluten meal has double the protein of SGF and, depending on the sorghum variety, a greater tannin content [19]. In brief, BG and DDGS with solubles are two types of cereal grains that are used for animal feed as well as industrial and human purposes. It includes minerals, fibre, protein, and fat, despite being less easily digested. Gluten-containing meals and feeds are byproducts of the starch-producing process.

3.2 Alternative Plant Protein Sources in Pakistan: Grain Legumes or Pulses

Pulses are grain legumes that are farmed mainly for their seeds, which may be utilized for industrial oil production, animal feed, or human consumption. Despite having comparatively low amounts of methionine, these legumes are prized due to more protein and important amino acid concentration [19]. Because of their high protein (25-33%) and starch (40-48%) contents, beans (*Vicia faba*) are beneficial for feeding humans and animals. They are similar to peas but have greater protein content and intermediate fibre levels (7-11%). They stand out for having a profile of low sulphur amino acids (0.6-1.0% methionine) and high lysine (5.4-6.8%). Since Faba beans mostly consist of linoleic and linolenic acids and contain 1% lipids, they may become rancid if left in the ground for a lengthy amount of time [25]. These are also glycosides, vicine and convicine, tannins, and lectins that are normally involved in unfavourable health reactions known as favism in people who are sensitive to glucose-6-phosphate dehydrogenase [19]. The pulses known as chickpeas originated from the species *Cicer arietinum*, belonging to the Fabaceae family, and related to *Vicia faba*, because of their high protein content, chickpeas are among the oldest cultivated pulses. Lysine contents are approximately 6-7% of the total protein; this is a very high percentage. Ruminant animals depend on bacterial synthesis while non-ruminant animals may be supplemented with threonine and amino acids which contain sulphur. Chickpeas, which constitute a low-fat level presumably below 5 %, contain several secondary metabolites that can reduce the capacity of the GIT to assimilate nutrients. Chickpea seeds may have varying levels of trypsin and chymotrypsin inhibitors, which might lower their usefulness as fodder for chickens. Chickpeas possess extra anti-nutritional elements in tannins and oxalic acid form [26]. Grown for its beans, which are used as food, feed, and fodder, lupines (Lupinus spp.) include white, yellow, and blue kinds. Lupine beans are prized for their excellent nutritional content, including around 35% protein. But they also contain alkaloids, namely quinolizidine alkaloids, which are important anti-nutritional factors that reduce feed intake resulting in bitterness, which can have neurophysiological consequences like convulsions and tremors. Lupines, compared with *Vicia faba* and peas, have modest amounts of saponin and little trypsin inhibitor action [27]. Because of more protein level (around 22-24%), peas (*Pisum sativum*), which are mainly farmed for human consumption, are a better substitute for soybean meal (SBM). It is more in lysine but less in tryptophan and amino acids containing sulphur [28]. In organic cattle husbandry, peas are advantageous since they provide protein in situations when SBM is unavailable [29]. Peas include anti-nutritional elements like tannins, lectins, phytate, as well as protease inhibitors [30]. *Cyamopsis tetragonoloba*, often known as guar, is a tall legume that has rose- or white-coloured blooms and trifoliate leaves. Foods often employ galactomannan gum as a thickening agent. Guar meal, as the primary by-product, has fewer sulphur and lysine amino acids than soybean meal, but it is still high in protein. Anti-nutritional elements may impede development and digestion, such as gum residue and other chemicals [31-34]. In brief, pulses are grown for their elevated protein and essential amino acid composition; instead, their methionine level is rather low. They are cultivated for human food, animal feed, as well as industrial oil production. Guars, peas, lupines, Faba beans, and chickpeas are all beneficial legumes, but since they contain anti-nutritional ingredients, they may become rancid and pose health risks.

3.3 Oil Seed and Fruit By-Products as Alternative Plant Protein Sources in Pakistan

The excellent by-products of oilseed crops, such as cakes or high-protein meals, are essential for feeding poultry. Although canola and soybean have received a great deal of research, lesser-known alternatives also contribute significantly to the protein composition of poultry diets [19]. The remaining meal is used as feed material after the oil is extracted. Oil is extracted by crushing fruits and oilseeds, and the leftover material is used as a feed component. The pericarp and seed of the oil palm fruit are the sources of several kinds of oil. The leftovers left over following seed extraction are useful, even if the pericarp pulp is not as good for feeding. The pericarp of a coconut is oil-free, but the seed produces a residue that looks like palm kernel meal when oil is extracted [19].

The cottonseed meal's (CSM) protein content differs depending on how well the oil is extracted and dehulled assessed. Between 25% and 5% of the total amount is crude fibre, while between 30% and 50% is protein. The residual oil in CSM varies greatly due to different extraction techniques; some meals have less than 2% oil residue, while others have more than 20%. In comparison to soybean meal, the reduced lysine concentration of CSM (4% vs. 6%) impacts the amino acid balance overall. When CSM isn't made from glandless seeds, gossypol is present, limiting its usage to non-ruminant animals like poultry. Iron salt supplementation is one way to reduce the toxicity of gossypol [35]. The by-product of linseed oil manufacturing, linseed meal, has drawn interest due to its polyunsaturated fatty acids, like conjugated linoleic acid and alpha-linolenic acid, used for improving the nutritional profile of cattle products for advantages to human health. But compared to other oilseed meals, it is deficient in methionine and lacking in lysine. Its high dietary fibre concentration prevents it from being incorporated into poultry diets [19].

The annual plant known as sesame (*Sesamum indicum* L.) reaches maturity in 70-150 days and reaches heights of 0.5-2.5 meters. Each of the two to four chambers in its elliptical pods has around twenty seeds. A meal made from sesame seeds that has undergone solvent extraction has 45-50% protein and less than 3% fat. Phytic acid, non-starch polysaccharides, and tannins are the major anti-nutritional factors in this meal. Sesame meal's high usage in diets without phytase supplementation might negatively impact the bioavailability of calcium and phosphorus due to its high amounts of phytate, which can reach 60 mg/g meal and bind around 80% of total phosphorus []19. The sunflower is the fifth-largest oilseed crop worldwide, accounting (8%) total output (FAO, 2011). Sunflower meal is extracted mechanically or with a solvent from whole or decorticated seeds. Processing techniques and plant attributes determine its quality [36]. Non-starch polysaccharides and crude fibre are abundant in sunflower meals, although lysine availability is restricted. Growth is impacted by hydrolytic enzyme inhibition caused by sunflower meal's 1.2% chlorogenic acid content. Its effects on chicken diets may be mitigated by adding supplements of methionine and choline. The percentage of protein might vary depending on the extraction technique, from 23% to over 40%. Meals that are not dehulled have a fibre content of 27-31%, whereas those that are dehulled or partly dehulled have a fibre level of 20-26%.

Depending on the extraction process, the residual oil content of coconut meal, which is made from dried coconut kernels, may range from 1 to 22 percent. With a high crude fibre content and low protein digestibility of lysine and methionine, it lowers the amount of energy that chickens can metabolise. Its high oil content makes it susceptible to rancidity. Of the non-starch polysaccharides, contains, mannan and galactomannan (25-30%) are particularly noteworthy since they have been shown to have anti-nutritional effects in non-ruminant animals [37]. Groundnut meal, also known as cake, is the leftover material following the oil extraction of groundnut seeds. Its protein and fibre content varies depending on how the hull is processed. Fertilised fruit produces a low-fibre, high-protein meal that is excellent for poultry feed. However, it has lysine and methionine deficiency, and it may get contaminated with aflatoxin, especially when stored in warm, humid circumstances [19]. When it comes to nutrition, palm kernel meal (which is often included in ruminant diets) is inferior to big oil meals. Its nutritional value is lower than that of soybean meal and other meals because of more crude fibre level (14-28%) and protein levels (14-20%), which are lower than those of copra meal. When mechanically extracted, it usually has a high oil content (6-15%). Though little prevalent, solvent-extracted palm kernel meal has a little greater protein level (19% vs. 17%) but a lower oil content (approximately 3%) [38].

In brief, poultry is fed with high-protein cakes or meals made from oilseed crops as byproducts. These leftovers are used as feed material after the oil is extracted. There are many types of oil produced by the pericarp and seed of the oil palm fruit. The nutritional value of groundnut meal and palm kernel meal is low, sunflower meal contains more crude fibre and non-starch polysaccharides, and sesame meal is deficient in both lysine and methionine. Protein-rich coconut meal is highly valued but is expensive.

3.4 Limitations on the Utilisation of Different Protein Sources

Anti-nutritional factors (ANF) are included in the composition of the majority of alternative protein sources contain ANF, which poses a serious challenge. Although traditional products also have these characteristics, alternative compounds have not received as much attention in terms of reducing them. The nutrient's digestibility and adsorption are hampered by ANF, protease inhibitors, phytate, lectins, polyphenolic compounds, glucosinolates, and saponins [19]. Paradoxically, feedstuffs that are often consumed for their protein content, including oil seed meals and grain legumes, also frequently have the greatest amounts of ANF. Soybean meals have a variety of ANFs, mostly heat-sensitive, and are thus neutralised in processing. However, these drugs have the potential to negatively impact animal physiology if left untreated [19]. However, heat treatment results in Maillard reaction products denaturation and production, resulting in the protein quality impairment [39]. Dietary trypsin inhibitors, often present in these sources, interfere with natural proteases to limit the protein's digestion, leading to pancreatic hypertrophy as well as growth suppression. Additionally, trypsin inhibitors, which are high in amino acids that include sulphur, may cause stress and methionine insufficiency, which is a major amino acid limiting factor in soybeans and a variety of substitute foods [19].

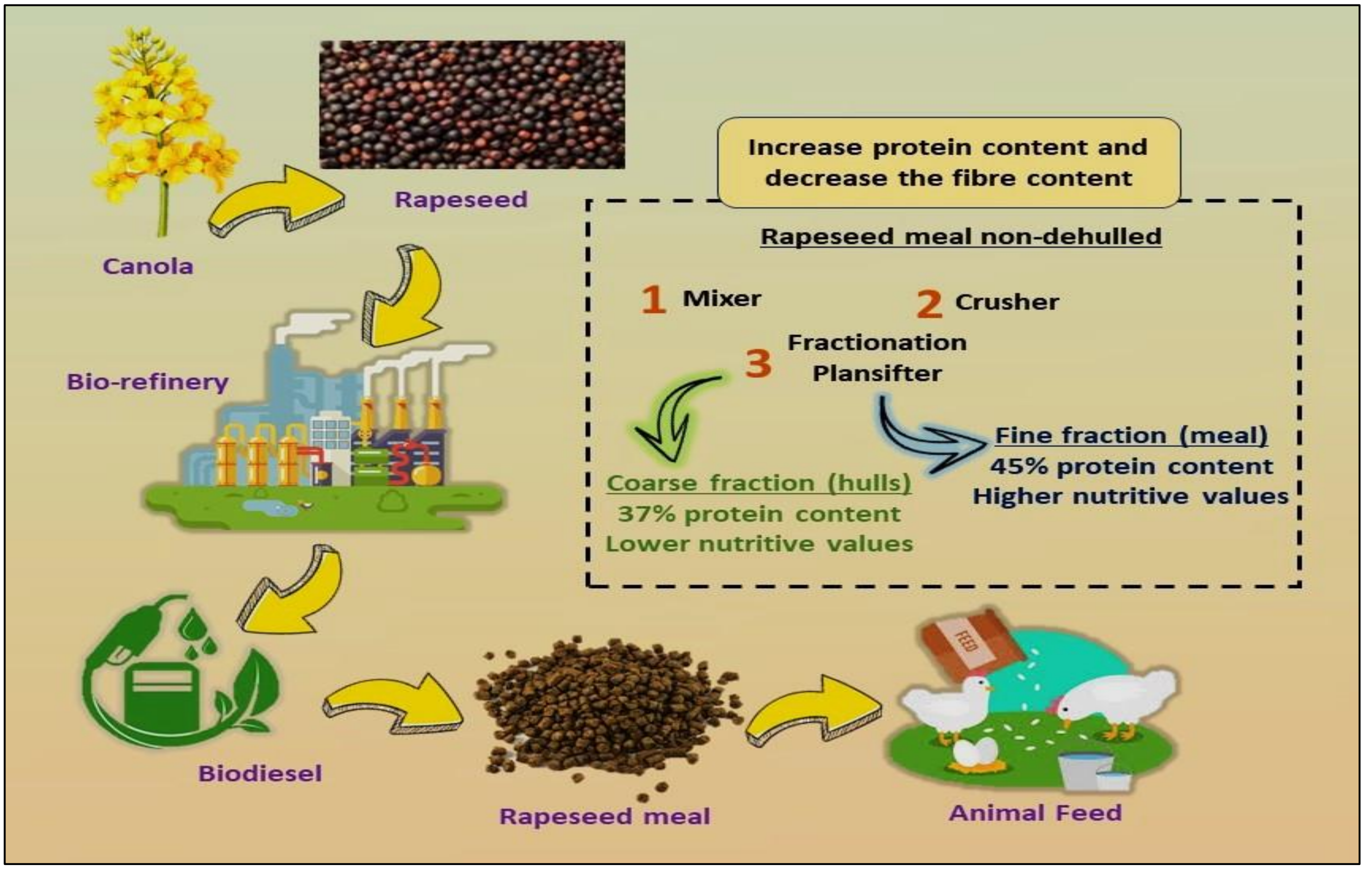
This has encouraged researchers to explore phytate's potential to bind minerals, proteins, and carbohydrates due to phytate's unique structure, which also hinders digestion, and reduces bioavailability of nutrients [40]. Phytotate can lower dietary protein utilization since it forms complexes with proteins or protein-starch which are hardly degradable by proteolytic enzymes [41]. Moreover, phytate also inhibits the activity of some intestinal proteolytic and amylolytic enzymes due to its chelating ability for multivalent cations [42]. Gossypol (polyphenolic leptoglobin) is contained in cotton seeds and adheres to dietary iron, thus has formed the practice of giving an egg yolk a brown residuum in birds as well as causes anaemia in birds [43]. Additionally, while ingested directly, could bind to lysine during processing, thus decreasing the protein quality. Most ruminant animals can handle gossypol through the rumen, however, other animals that are classified as non-ruminant animals are prone to the toxicity of gossypol. To reduce and neutralize the effects of gossypol in the cottonseed meal, different processing methods have been developed [44].

Lectins, or glycoproteins, attach to brush-border glycosyl units bind to specific sugar residues, and also withstand intestinal proteolysis [45]. Lectins agglutinate animal erythrocytes, induce lymphocyte mitosis, and attach to the intestinal mucosa, hindering the absorption and digestion of nutrients. They also decrease the utilisation of protein to an even further extent because they interfere with the activity of the digestive enzymes. Phenolic chemicals are capable of binding vitamins and minerals and were shown to slow the digestion of protein and carbohydrates. Some of these could harm the mucosa of the digestive system and also interfere with enzyme activity, consequently leading to poor nutrient absorption like vitamin B12 [19]. The velvet bean var. Utilis, is one of the identified legumes found in the tropics and subtropics and has several uses such as medicinal and ornamental. This plant species falls under the family of Fabaceae and possesses diverse medicinal uses. This characteristic of high phenolic may bear implications for utilization as opposed to other cultivated pulses [46-48]. Such restraints include underproduction and availability of substitute feed components. Since most of these components have substantially lower yields in terms of land area and are consumed for cultivable food or even for wild plants where breeding alterations have not been done, the majority of them have insignificant downstream usage in the animal feed and agriculture market [19]. In brief, because of specific anti-nutritional substances present in unconventional protein, the effectiveness of absorption and utilization of protein decreases. Phytates, lectins, polyphenolic chemicals, glucosinolates, and saponins are several substances that may harm animal physiology and cause hypertrophy and growth inhibition in the pancreas. Despite this, phytate has a structure that facilitates the inhibition of nutrient absorption.

4. Evaluation of Rapeseed Meal as a Promising Alternative

According to Li et al. [49], rapeseed has a protein level of 17% to 26%. Between 2000 and 2015, a protein made up 37.0% to 41.4% of meals prepared in Canada at 12% moisture content [49]. Rapeseed proteins are mostly of a storage nature, making up around 38% [50]. These proteins are found in crystalloid structures and protein storage vacuoles inside the matrix [51]. Cruciferin and napin are major storage proteins in rapeseed; their molecular weights have been reported to be 300-350 kDa and 12-16 kDa [17], around 300 kDa and 14 kDa [51], and roughly 300 kDa and 12-14 kDa [14]. The ratios of these proteins vary between 0.6 and 2 in European variants. In addition to storage proteins, *Brassica napus* has structural oil body proteins and metabolic proteins, such as lipid transfer proteins, which may be removed from the seed or meal during the processing process [17, 52]. The process of enhancing the protein content and reducing the fibre content in rapeseed meals for use as animal feed is illustrated in Figure 1. The process begins with rapeseed (also known as canola) which is sent to a bio-refinery for initial processing. At the bio-refinery, the rapeseed meal undergoes three main steps: mixing, crushing, and fractionation through a plansifter. This sequence results in the separation of the meal into two fractions: the coarse fraction (hulls), which contains 37% protein and has lower nutritive values, and the fine fraction (meal), which contains 45% protein and has higher nutritive values. Additionally, biodiesel is produced as a by-product during this process. The fine fraction, which has a higher protein content, is ultimately used as animal feed, providing a nutrient-rich diet for animals.

Figure 1: Rapeseed meal as sustainable feed



4.1 Replacement Values of Rapeseed Meal to Other Protein Sources

There were no negative impacts on the development and well-being of developing rabbits when 80 g/kg of soybean meal was replaced with mustard cake [53]. Furthermore, de-oiled rapeseed meal seems to be a capable substitute for groundnut cake in poultry diets [9]. Without compromising the performance of the broilers, rapeseed meal may make up to 25% of complex feed compositions [54]. Total dry matter intake, total body weight gain, and me intake (kcal/day/bird) decreased with increasing amounts of rapeseed meal when it was substituted for soybean meal in broiler starter and finisher rations at different ratios. Total dry matter intake and total body weight gain decreased as the rapeseed meal amount in the diet rose, a tendency also seen in broiler finishers [55]. The liver size and the relative weight of the gallbladder improved in male broiler chicks when 25% of their soybean meal was replaced with rapeseed meal. Therefore, the research concluded that broiler chick performance could be maintained even if 25% of soybean meal was substituted with rapeseed meal in their diets [56]. In brief, cruciferin and napin contain 90% of the 26% of the protein that is present in rapeseed. Other proteins include those that are structural and metabolic components of the oil body. Furthermore, without sacrificing performance, rapeseed meal may be added to broiler compound feed formulations, up to 25% of the total. Increased rapeseed meal intake is associated with a larger liver and gallbladder, and overall body weight gain.

4.2 Rapeseed Cultivation and Processing in Pakistan

Important oilseed species that are grown in Pakistan include mustard and rapeseed. They are prized for their high oil content, which ranges from 40 to 46%, and their high protein content, which is between 38 and 40%. Canola varieties or hybrids, which are highly prized for their appropriateness for human consumption and as animal feed, are defined as types of rapeseed and mustard that have less than 2% erucic acid in oil and less than 30 umole/g glucosinolate in oil-free meal. With an annual output of 102.0 thousand metric tonnes as of 2018–19, Pakistan boasts extensive cultivation of these crops, spanning over 26.02 thousand hectares. Rahim Yar Khan, Muzaffargarh, Bahawalpur, Bahawalnagar, Attock, Rawalpindi, Jhelum, Chakwal, Faisalabad, and Multan are among the major agricultural areas. Over the years, several cultivars have been produced at the Oilseeds Research Institute in Faisalabad; each has unique qualities and is suitable for a variety of environments [57].

The intercropping with the September sown Raya is possible with Anmol Raya which was developed in 1988 and is a high-yielding thermo-tolerant and pest resistance variety. There are also other varieties of canola which are popularly known as Punjab canola, Faisal canola, AARI canola, and Rohi Sarson also come with their advantages such as high yield, heat and drought tolerance, and are used in inter-sown crops. Similarly, BARI in Chakwal has developed its variety like Chakwal Sarson, Chakwal Raya, Barani Canola, Barani Sarssoin, RD-73, and Barani Star (Rapeseed) associated with Barani regions of Punjab. These varieties differ from the others by their higher yield, better adaptation to growing conditions and disease and drought resistance, and lodging [57]. In brief, Pakistan is a country that grows mustard and rapeseed oilseeds, which are prized for their increased oil and protein content. It is beneficial to employ canola hybrids and cultivars for both human consumption and animal feed.

5. Nutritional Composition of Rapeseed Meal

Because of its higher protein level (30-40%) and significant concentrations of lysine, methionine, and tryptophan, rapeseed meal is especially valuable as a source of protein [58-59]. Rapeseed meal has a comparable, and sometimes even superior, amino acid balance to soybean meal while having a lower protein digestibility (72% vs. 88%) [9]. This is especially true for sulphuric amino acids. The literature included in Table 1 is in the late 20th century, which is the period of the late 20th and early 21st centuries. Even though up to 65% of the phosphorus in rapeseed meal is bound in P-phytate, which reduces its bioavailability, the meal is nonetheless rich in calcium, phosphorus, manganese, selenium, and iron [58]. A unique vitamin composition of rapeseed meal is its high nicotinic acid and choline content, along with low amounts of carotenoid and vitamin D [10, 60]. Rapeseed meal's amino acid composition is similar to soybean meal's, with the notable difference being the higher methionine content [9]. Table 2 presents research findings on heat treatment, digestibility, and protein utilisation from studies and research conducted primarily in late 20th century. It also covers factors affecting the nutritional value of rapeseed meal.

Table 1: Insights into the variability and quality of rapeseed meal protein, as well as factors influencing its digestibility and nutritional value.

Studies	Key Findings
Bell, [61]	Reported average crude protein: 42.8%.
Blair et al. [62]	Protein contents for varieties Triton (46.9%), Westar (42.9%), and commercial canola (41.0%).
Clandinin & Robblee, [63]	The protein level of rapeseed meal from *B. napus* (38-39%) is higher than from *B. compestris* (35%), while commercial meal averages 36-37%.
Clandinin, [64]; Zeb, [65]	Average protein content of low glucosinolates varieties: 37-38%.
Durrani & Khalil, [66]	Reported crude protein values: *B. juncea* (41.5%), *B. napus* (37.6%), *B. carinat* (35.8%).
Fernandez et al., [67]	Protein solubility decreases in oilseed meals with increased autoclaving time.
Finlayson, [68]	The proposed conversion factor for nitrogen to true protein is 5.53.
Grala et al., [69]	Increased toasting temperature decreases lysine contents of rapeseed meal.
Henkel & Mosenthin, [70] Zeb, [65]	Protein content categories: low (36.3%), middle (39.8%), high (43.7%).
Jensen et al., [71]	Toasting decreases protein solubility and true digestibility of rapeseed meal.
Keith & Bell, [72]	Heating rapeseed meal affects lysine availability, reducing its nutritional value.
Khorasani et al., [73]	Acid solutions reduce the in-vitro digestibility of rapeseed meal.
Kohnhorst et al., [74]	Heating improves in-vitro protein digestibility in common dry beans.
Lin & Lakin, [75]	Heat treatment improves in-vitro protein digestibility in soy meal.
Rogulski, [76]	NPN in rapeseed meal consists mainly of low-molecular peptides, glucosinolates, and free amino acids.
Rogulski, [76]	The nutritive value of the NPN fraction extracted from rapeseed meal is low.
Sandmann & Schon, [78] Zeb, [65]	Rapeseed meal serves as a protein supplement in poultry rations, rated to be of high quality.
Zeb, [65]	Variation in amino acid composition and non-protein nitrogen renders %N x 6.25 inappropriate for estimating true protein.

Table 2: Summary of Research Findings on Rapeseed Meal Amino Acid Composition and Nutritional Implications.

Reference	Description
Bell, [61]	Focusing on amino acids and their physiological availability in addition to crude protein is still the most significant nutritional strategy, even though the proper conversion factor of nitrogen to protein value can have significant effects on protein quality, digestibility, and nitrogen utilisation.
Fenwick & Curtis, [79]	It is possible to note significant differences in the various amino acid compositions of rapeseed meal. Differences in agroclimatic conditions, oil extraction methods, genetic backgrounds, and analytical methodologies may all be used to explain these variances.
Grala et al., [69]	Toasting decreases the lysine content and protein value of rapeseed cake in rats, with temperature having a more significant effect than heating time.
Jensen et al., [71]	Total lysine content in rapeseed meal drops after heat treatment, with other amino acids remaining unchanged. Glucosinolates and the nutritional value of rapeseed meal are affected by heat treatment.
Leslie & Summers, [80]	Rapeseed meal's amino acid balance is affected by lysine supplementation, with arginine and methionine also playing roles.
Lin & Lakin, [75]	Heating treatments of proteins result in losses in lysine content. Heating with steam and under atmospheric pressure causes lysine losses only when the heating time exceeds 40 minutes. The Maillard reaction and other reactions during heating contribute to lysine loss.
Näsi & Siljander-Rasi, [81]	Thermal processing effects on digestibility and protein utilization of rapeseed meals are similar among various cultivars and differently processed rapeseed meals, with different glucosinolate levels.
Barbour & Sim, [82]; Muztar & Slinger, [83]; Muztar et al., [84]; Nwokolo et al., [85]	Essential amino acid presence in rapeseed meal ranges from 69% to 96%, with lysine having an overall availability of 90% in chicks.
Nwokolo et al., [85]	The availability of essential amino acids in rapeseed meal ranges from 69% to 96%, with lysine having an overall availability of 90% in chicks.
Shahidi et al., [86]	Efforts to increase the nutritional quality of rapeseed meal comprise treating it with ammoniated methanol, aimed at reducing glucosinolates and other anti-nutrients, without adversely affecting essential amino acid contents.
(Summers & Leeson, [87]	Amino acid imbalances are observed when rapeseed meal is the sole dietary protein source, but not in practical diets where protein comes from multiple sources.
(Summers & Leeson, [88]	Lysine in canola meal is 90% as available as that in soybean meal, while methionine availability was estimated to be similar in both meals, based on gain and feed: gain response in chicks.
(Summers, Bedford, et al., [89]	Calcium and sulphur supplementation interactions affect the performance of broiler chicks fed rapeseed meal diets.
(Summers, Spratt, et al., [90]	Ca and S supplementation interactions affect the performance of broiler chicks fed rapeseed meal diets, with no alterations in liver weight, suggesting little or no liver involvement in reduced performance.
(Summers et al., [91]	Similar mineral imbalances are reported in rapeseed meal feeding to poultry, affecting performance but not liver weight.
(Zuprizal et al., [92]	True digestibility of amino acids in whole and dehulled rapeseed meals differs, with dehulled rapeseed meals having higher lysine availability.

In brief, rapeseed meal is less digestible than soybean meal, it nonetheless has a high proportion of protein and an excellent amino acid balance. It has high quantities of choline and nicotinic acid and is rich in calcium, phosphorus, selenium, iron, and manganese. From Table 1 it was concluded that the quality and diversity of proteins in rapeseed meal are determined by genotype and agro-climatic conditions. It is added to chicken feeds as an additional source of protein. Heating, however, changes the availability of lysine and lowers its nutritional content. The protein composition is also influenced by elements such as free amino acids, low-molecular peptides, and glucosinolates. The amount of lysine is reduced at higher toasting temperatures. Moreover, from Table 2, it was concluded that the necessary amino acids found in rapeseed meal range from 69% to 96%, with chicks having 90% availability of lysine. Supplementing with arginine and methionine affects the equilibrium of lysine. Although lysine content may be decreased by heat treatment, total lysine content decreases. The impact of thermal processing on protein utilisation and digestibility is consistent across different processed rapeseed meals and cultivars. Interactions between calcium and sulphur supplements on broiler chick performance.

5.1 Feeding values of rapeseed meal

Rapeseed meal serves as a common feed component for various livestock and aquatic animals, yet determining precise feeding proportions poses challenges due to several factors [59]. Mustard cake, when fed to sheep and goats, has been observed not to significantly affect various parameters including feed intake, efficiency, nitrogen and mineral balance, and growth performance in growing lambs [59]. However, feeding fish with mustard cake at 20 to 30% levels resulted in liver abnormalities, congestion, and cytoplasmosis in Indian major carp, suggesting an optimal inclusion rate of 10% [93]. Un-detoxified rapeseed meal containing antinutritional agents poses risks in chicken rations, potentially leading to severe struma and liver bleeding, with common rapeseed meal typically avoided in diets for young chickens [60]. For broiler chicks, inclusion levels of up to 10-15% may be feasible, while laying birds may tolerate 7.5-8% rapeseed meal in their diet; however, higher inclusions, such as 12%, could reduce egg weight and hatch rates [60]. High-glucosinolate rapeseed meals have been linked to iodine deficiency disorders in young chickens [60]. Mustard cake inclusion in layer diets showed adverse effects on egg weight, shell thickness, and yolk quality at 15% inclusion, with deterioration in albumen quality even at 10% inclusion [9]. To ensure safety, rapeseed meal inclusion should maintain total glucosinolate levels below 1 to 1.5 mmol per kg of diet, with varying tolerances observed depending on glucosinolate types, animal age, and species. Ruminants generally tolerate higher levels compared to monogastrics, yet inclusion above 10 mg/kg of total glucosinolates have been deemed toxic to pigs and poultry, associated with poor growth and reduced thyroid hormone levels in ruminants [9]. In brief, mustard cake affects feed intake, efficiency, and growth performance, it might be difficult to determine the feeding quantities for livestock and aquatic animals, even if rapeseed meal is a frequent feed component.

5.2 Digestibility

The term "digestibility" refers to the ratio of an animal's nutritional intake to its excretion, expressed as faeces or terminal ileum output, divided by the number of nutrients consumed [94]. Unlike measuring the absolute nutrient absorption over an animal's lifetime, digestibility assesses the percentage of nutrients disappearing during passage through the digestive tract. Feed formulation and nutrient recommendations often rely on digestibility as a crucial method for estimating nutrient utilization [94]. However, it's important to recognize that digestibility reflects the proportional utilization of nutrients from feed over a specified period. To ensure robust digestibility responses, especially in enzyme efficacy tests, data on intake, growth attributes, body composition, and bone ash should be provided to support digestibility coefficients. Protease, phytase, mannanase, pentosanase, cellulase, β-glucanase, pectinase, and amylase are among the eight enzyme activities capable of breaking down protein, phytate, mannan, pentosan, cellulose, glucan, pectin, and starch, thus enhancing nutrient absorption and digestion in birds' intestines [95]. Broiler chicks fed diets supplemented with enzymes exhibited increased apparent digestibility of dry matter, crude protein, and ether extract compared to untreated controls [96]. Adding commercial enzymes to chicken feeds containing sunflower boosted fibre digestion, mitigating adverse effects [97-98]._Xylanase addition reduced competition among gut microbiota for nutrient utilization, resulting in improved nutrient retention in birds fed wheat-soybean meal diets [99]. Multi-enzyme supplementation significantly improved nitrogen digestibility in broiler chickens fed corn-soy diets [100]. The addition of enzymes to different feeds improved all the parameters related to digestibility and nitrogen retention when compared to feeds where no enzymes were added [101]. The use of multi-enzyme complexes in feeding has been found to alleviate energy and nutrients, especially in diets with low energy density [102].

An antioxidant apart from phytogenics is the supplemental enzymes such as alpha-galactosidase which increases the energy digestibility of the broiler diet [103]. Supplementation with Avizyme helped decrease fresh faeces output, and improved dry matter digestion and nitrogen retention in birds [104]. There is a positive effect of supplementation of exogenous enzymes among them β-glucanase when broilers were fed on the wheat–barley–soybean meal-based diets [105]. Multiple-enzyme additives increased the dietary digestibility of matter and dry organic matter wheat-soybean diets [106]. However, enzyme supplementation on nutrient digestibility differs with the species of the bird, the dietary fed to the bird, and the type of enzyme to be used [107-109]. Some findings feeding protease supplementations influence amino acid digestibility [110-113]. Several studies reveal that enzyme addition particularly phytase, improved the factors like dry matter, gross energy, crude protein, calcium, and phosphorus in birds [114-116]. Studies have further shown that various factors such as the age of birds, period of the experiment, protein source in the diet, or type/origin of the enzymes can affect the digestibility values, which indicates that the interaction of the enzymes with nutrients can be a very complicated process [117-119]. In brief, it is an essential requirement when it comes to feed formulation and suggestions of nutrients as it aids in the determination of the nutrient use. There are many enzyme activities, such as protease, phytase, mannanase, pentosanase, cellulase, β-glucanase, pectinase and amylase that might have beneficial effects for nutrition absorption and digestion in the intestines of birds. Nevertheless, enzyme supplementation could improve the nutritional digestibility of birds foraging on meals, based on some variables like bird species, feed content, and the enzyme type used

5.3 Allergenicity

In the past fifteen to twenty years, the reported incidence of allergic diseases which is linked to food allergens has been on the rise and the estimated incidence is 1.5-2%. This amount costs from 5-2% of adults and 5-8% of children in developed countries. Plant-source allergens are especially because of seeds of the Brassicaceae family, especially 2S albumins because of their extremely stable tertiary and quaternary structures which are regarded as terribly allergic [52, 120]. Mustard, a major food allergen from the Brassicaceae family, shares similar allergenic properties with 2S rapeseed albumins [52, 121-122]. Cross-reactivity exists among different Brassica genus 2S albumins, such as those in *Brassica napus*, *Brassica juncea*, and *Brassica nigra* [123-124]. Sensitization to *Brassica napus* and *Brassica rapa* has been linked to allergic rhinitis, asthma, and sensitivities to pollens and foods, especially in children with atopic dermatitis [125]. Additionally, cruciferins may also possess allergenic properties [126].

Recent studies have identified major mustard allergens in mustard seeds, including Sin a 1, Bra j 1 (albumins 2S), and Sin a 2 (globulins 11S). Moreover, new IgE-binding protein bands have been discovered in *Brassica juncea* and *Sinapis alba* varieties, such as β-glucosidase, oleosin, Glutathione-S transferase proteins, and enolase [127]. Though napins and cruciferins may be found in cold-press rapeseed oil, further research is needed to understand the clinical significance of exposure to these proteins at low concentrations [125]. European Commission regulations mandate appropriate labelling of food products containing rapeseed protein due to their potential to trigger allergic reactions in individuals sensitized to mustard and its derivatives [128]. However, some studies suggest that labelling rapeseed-containing foods as potentially cross-reactive might not be entirely accurate, as practical instances of cross-reaction between mustard and rapeseed remain undocumented. This inconsistency in labelling practices could confuse diet professionals and consumers [129]. In brief, western societies must recognize food allergies, especially 2S albumins part of seeds in the Brassicaceae family. The possible allergens in mustard seeds include Sin a 1, and Bra j 1, Sin A 2. As the European Commission rules require that foods containing rapeseed or rapeseed oil must be labelled properly, this leads to the fact that there are various ways of doing it which just confuses the customers.

5.4 Functionality

Individual functionality of proteins is of great importance for food formulations, since proteins play essential roles in foods. Besides being major nutritional factors they also play a structural role in foods, influence the bio-accessibility of several compounds, and appear to be appreciated by consumers because many may associate them with some pleasure when eating. However, proteins could at times be destructive or negatively impacting this is why it is important to take a balanced approach regarding the proteins. These properties may vary according to the proteins inherent in the protein the means and method it underwent through the production process and the conditions that the proteins are exposed [130-132]. Therefore, rapeseed protein isolate can be considered a valuable functional food component, studying which mentioned characteristics such as solubility, emulsifying capacity, and the ability to foam, will contribute [133].

5.4.1 Solubility

Protein solubility is one of the fundamental properties of protein dispensing and it has been found to correlate strongly with other desirable functional properties of proteins such as emulsifying, foaming, and gelation properties of protein-derived products [133]. From the previous study carried out on the rapeseed protein isolate, solubility depends on such factors as pH level, temperature, and salt content in the case of cruciferin and napin proteins [17]. Functionality and solubility, two closely related aspects, mainly depend on the molecular make-up of proteins. The influence of solubility is thus revealed to have been affected at the secondary and especially the tertiary structure of rapeseed protein through a measure of ultra-sonication. Ultrasound can modify protein location and molecular arrangements since it deforms bonds that are secured by electrostatic force or hydrogen bonds causing an increase in the openness of the protein chains. The FTIR results revealed an increase in β-turn and β-sheet contents in addition to suppression of random coil, and α-helix contents thus enhancing solubility [49].

Cucurbitins seem to dissolve well in strong acidic surroundings whereas, napins do not dissolve in strong alkalis On the other hand, cruciferins remain soluble in strong alkalis as well as in strong acids [134]. Further, the treatment of seed storage proteins of tested samples revealed an increase in solubility when NaCl and $CaCl_2$ were introduced into the extraction medium. The solubility of cruciferin and napin was also studied, and the results showed that solubility was influenced by the change in pH and NaCl concentrations. According to the observed trend for cruciferins, solubility was found to be maximum at pH 3. Thus, at 0 mM NaCl, it is evident that the enzyme tends to be inhibited or retarded in its activity due to the presence of NaCl, regardless of the NaCl level, signifying a salting-in effect of NaCl. On the other extreme, the result showed that napin solubility was very stable and stayed above 90% in all of the experiments carried out and did not decrease due to a change in concentration of NaCl as demonstrated by experiments done [135-136].

Researchers were also able to determine the solubility of proteins concerning the method of protein isolate production. For instance, although the canola protein isolate extracted through the aid of salt was found to have a solubility of approximately 80%, the same solubility reduced to below 5% at the end of the isoelectric precipitation process [137]. Analogously, the isolated canola protein through alkaline extraction and subsequent separation by precipitation techniques was found to be soluble in response to the pH variation with the highest solubility at higher pH values [138]. The research was aimed at determining the impact of sequential isoelectric precipitation on the solubility of two rapeseed protein isolates and it was deduced that the solubility of rapeseed protein depended on pH and salt. These isolates had two different soluble characteristics assuming the different fractional fraction for every single one of the isolates which has responded differently to NaCl supplementation about pH and kind of isolate [139]. In brief, protein dispersions are characterized by solubility, and solubility is defined as changes that are elicited by factors; for instance, pH, temperature, or salt. On comparing the solubility of cruciferin and napkin, while analysing parameters of isolation of protein from rapeseed, it came to the observation that it is not the same. Likely, the use of ultrasonic treatment of protein molecular structures will result in the formation of new states of protein functional properties at the molecular level and a positive change in solubility, while the processing conditions influence solubility.

5.4.2 Emulsifying properties

Researchers examine the emulsifying activity of cruciferin protein isolate and napin protein isolate and the effect which stems from the alteration of the molar concentration of NaCl (0mM, 50mM, and 100mM) and pH (3.0, 5.0, 7.0). They investigated the emulsifying activity coefficients of these isolates, the EAI, as well as the constants of emulsifying stability, the ESI. EAI is defined by the extent of the interfacial area that was hit by the emulsifier during emulsification, surface activity index. At the same time, ESI sheds light regarding the stability of the emulsion, formed at the given conditions. pH and NaCl concentration caused the EAI of cruciferin and napin isolates and the interaction to a significant extent were reflected through the results obtained. Concerning cruciferin protein, the EAI values trend revealed that the decrease in the protein solubility occurs when the NaCl concentration was raised at pH 5.0 and 7.0. The value for protein was more at the physiological pH of 7.0 and the least at 3.0. From this study, it was also observed that as hydrogen bond rises, the emulsifying activity also rises because the proteins get a chance to align at the interface thus; there exists little interfacial tension. In the same way, all three napin isolates possessed the same characteristics of emulsifying but the emulsifying properties were observed at the range of pH 3 up to pH 7.0 and 5.0. As in similar experiments, an increase in the concentration of NaCl was observed to enhance the effectiveness of the system within the range of provided pH values. Another spectacular idea outlined in the research was the interaction between the surface charge and solubility as factors defining the emulsifying properties of the napin isolate and its analogs, however, it is worth indicating that solubility might not be the only reason, which affects the emulsification properties of certain proteins [135-136]. In the same way, the emulsification capabilities of cru de canola globulin, cru de canola albumin, and canola protein isolate were assessed in varying pH environments. The obtained result also pointed out that globulin fraction of the three tested seed varieties possesses higher emulsifying capacity the particle size of which is comparatively smaller than other fractions. In the present study, globulin fraction remained unchanged as expressed by EAI when compared with the effect of pH while the result showed that EAI of albumin canola protein isolate significantly decreased when exposed to the effect of pH. It was explained that due to the high molecular weight polypeptides and disulphide bonds, the Canola protein isolate has low emulsifying efficiency, which could only allow it to be unfolded to provide stability to the emulsion [140].

The investigation into emulsion stability emphasized the above findings: While it can be appreciated that the fractionated canola protein was comparatively stable most of the time while undergoing the experimental procedures. This confirmed the effect of the CPA emulsifying properties due to the measures taken and the comparison carried out between the EAI and ESI of isolate from salt extraction with that of the isoelectric precipitation. Some of the reported book findings include: When the surface-active proteins were isolated through salt-extraction, the emulsifying activity and stability were significantly higher than those proteins which were isolated using isoelectric precipitation [137].

Furthermore, it explained the role of the kind of the starting material and heating treatment on the emulsifying ability of rapeseed protein. Protein once extracted from cold-pressed cake was found to possess a better quality of emulsifying potential than the protein available in hot-pressed and solvent-extracted meals though heating had a negative influence on the stability of the emulsion. This again supports that the processing conditions were instrumental in determining the functionality of the proteins [1]. In brief, the functioning of proteins largely depends on such parameters as emulsifying stability indices and emulsifying activity indices. Solubility, surface charge, pH sensitivity, and the presence of NaCl have an influence on the emulsifying activity index of purified napin and cruciferin. As a result, evidence indicated that the emulsifying properties of canola protein isolates varied and the functionality of the protein was influenced by processing temperatures.

5.4.3 Foaming properties

Foaming activity is attributed to a protein structure that enables them to be surface active with capabilities of decreasing the surface tension when adsorbed at interfaces of air and water. Foam formation occurs through four processes; diffusion, adsorption, interface, and unfolding while foam stability is the ability of foam to withstand mechanical or gravitational pressures [141]. The foaming ability of the different proteins varies and the results showed that the napin foams have excellent foam stability as compared to cruciferin foams. However, based on the findings, it has been revealed that the 2S and 11S rapeseed proteins possess great foaming characteristics (Wanasundara et al., 2016). The foam capacity and stability of canola protein isolate across different pH values (4.0, 7.0, 9.0) was explored. Concerning the effectiveness of canola protein isolate as a foam former and the foam stability index, the values were substantially investigated at different pH 4.0, 7.0, and 9.0. The highest foaming capacity was at pH 9.0 (66.91%), decreasing slightly at pH 7.0 (57.83%) and significantly at pH 3.0 (19.62%). Foam stability over 60 minutes was highest at pH 9.0 and lowest at pH 4.0, correlating with solubility and hydrophobicity. The balance of hydrophobic and hydrophilic groups influences foam stability, with insoluble proteins potentially preventing air bubble coalescence. Hydrophobicity appears more closely related to foam capacity than solubility. It's important to note that high foam capacity doesn't always translate to high foam stability, as different molecular features are required for each process [142]. The foaming capacity and stability of the canola protein isolate were determined and they were found to be relatively low (12.29 ml) foam-possibly due to the breaking of disulphide bonds during protein isolation [138]. In brief, rapeseed proteins may have different foaming properties and napin protein should be more stable than others. The pH affects foam stability; the maximum foam stability is observed at a pH of 9.0 and minimum at a pH of 4.0 and foam capacity is more affected by hydrophobicity rather than the solubility of the substance studied.

5.4.4 Gelation

Protein functional properties make them very important in food products such as thickeners and gels and the structure of foods like jellies, cheese, yogurt, sausages, tofu, and puddings. This is the capacity of the proteins in the system to create a matrix or an ordered third dimension that is capable of holding a reasonable quantity of water once both the repulsive and attractive forces are balanced. This process mainly involves the partial unfolding of the protein in question which changes its conformation and then the clipping of these molecules to form aggregates [1]. The effects of heating temperature and pH on gel properties of canola proteins were studied and cruciferin and napin, the fractions of canola protein were determined based on temperature (80-120 °C) and pH (5.0-11.0). In terms of gel and thermal properties, it was noted that cruciferin and napin isolates have different characteristics and these differences could have arisen from differences in amino acid profile, and molecular mass all in all cruciferin isolates gave better gel properties than napin isolates. Thus, Napin formed only weak gels at pH 5.0, most likely because at low pH and in the presence of acid, such as in the media, molecules partially unwind. At pH 7.0 there is a slight increase in the gel compressive stress with the increase in temperature. Napin gels showed the highest mechanical features at 120 °C and pH 9.0, possibly due to disulphide bridge breaking at basic pH, facilitating intermolecular interactions. Similarly, cruciferin gels exhibited high compressive stress at pH 11.0. Despite their potential in non-food applications like biosensors and tissue engineering, the extreme conditions required for optimal gel formation may limit their food applications [143].

Moreover, the gelling properties of canola protein isolates across different pH conditions (pH 3.0-9.0) were studied as stronger gels formed at pH 9.0 compared to pH 7.0, while gelation did not occur at pH 3.0. Differential scanning calorimetry revealed denaturation temperatures of approximately 78 °C: pH 5.0 and 87 °C: higher pH values (7.0/9.0), with gelation occurring at approximately 82-86 °C regardless of pH. Gel density increased with higher pH values, indicating the great gelling potential of canola protein isolates, particularly at pH 9.0 [144]. The effect of salt level and pH on the gel properties of canola protein isolate and its globulin and albumin fractions was studied. Increasing pH values improved gel properties such as elasticity, firmness, fracture stress, and overall deformation resistance. The addition of NaCl harmed globulin and albumin gel features but improved properties in canola protein isolate gels. Globulins exhibited better gelling performance compared to albumins and canola protein isolate, making them suitable for applications like sausages, soups, sauces, and jams [140]. The gelling abilities of rapeseed proteins by determining the least gelation concentration were also determined and canola protein isolate showed partial gel formation at 10% concentration and strong, stable gels at 14% concentration [138]. In brief, protein conformational changes and fragmented denaturation are involved in the gelation of canola proteins. Temperature and pH during heating are important factors, and cruciferin exhibits better qualities. Gel density is increased by strong gel formation at pH 9.0, and characteristics are enhanced by raising the pH. Rapeseed proteins exhibit robust, durable gels as well as partial gel formation.

6. Poultry Responses to Diets Containing Sustainable Protein Sources: Rapeseed Meal

The biological value and nutritional significance of dietary proteins rely on the presence and balance of essential amino acids. Adequate levels of essential amino acids enhance the nutritive quality of proteins [145]. However, beyond amino acid composition and digestibility, additional factors influence the biological value of alternative protein sources, often in a tissue-specific manner. Understanding these values is crucial in formulating diets for poultry, as it allows for greater inclusion of alternative ingredients, particularly those of lower quality, thus expanding the range of ingredients, enhancing formulation precision, and ensuring consistent bird performance [39]. Dietary protein sources are diverse mixtures of different proteins, each varying in biological value and capacity to fulfil animal protein and amino acid requirements. Processing methods further affect protein sources' suitability for poultry diets. Consequently, the response of poultry to diets containing various protein sources must be evaluated concerning nutrient profiles. Although soybean meal (SBM) is commonly used as a reference, alternative ingredients such as groundnut and fully dehusked sunflower meals can substitute SBM in poultry diets. For instance, cottonseed meal (CSM) is employed in low nutrient-density diets due to its inferior lysine content compared to SBM [146]. However, CSM supplemented with lysine-rich additives can replace a significant portion of SBM protein in broiler diets without adverse effects [19, 35].

Groundnut meal, while comparable to SBM, lacks balance in essential amino acids for poultry, particularly cysteine, and methionine. Studies suggest that groundnut meal can be effectively utilized in poultry nutrition with adequate dietary lysine and methionine levels [19, 147]. However, excessive heating during processing compromises groundnut meal's protein quality, as seen in many oilseed meals [19]. Similarly, high-oil sunflower seed meal can replace SBM in starter diets for young chicks, potentially meeting insoluble fibre requirements for enhanced gizzard function and broiler liability [148]. Linseed meal, akin to canola meal, is rich in protein and energy but deficient in lysine. While linseed meal supplementation increases ω-3 fatty acid levels in poultry products, excessive inclusion can reduce bird performance [149]. Additionally, flaxseed meal supplementation beyond 10% in laying hen diets can decrease egg production and increase liver haemorrhage incidence [150]. Seaweed products, including Chlorella and Spirulina algae, have been explored for their protein content and potential benefits on poultry performance. While some studies report improvements in feed intake and egg production, results are inconsistent, and further research is warranted [19, 151-152].

In brief, alternative protein sources are influenced by several aspects such as tissue-specific characteristics, digestibility, and content of amino acids. Comprehending these values is essential for diets using poultry since it permits the use of less-quality components. Soybean meal substitutes include groundnut and sunflower meals; nevertheless, overheating may reduce the quality of the protein. Additionally, using insects won't negatively impact growth performance.

7. Improving the Nutritive Value of Sustainable Protein Sources for Poultry

Efforts to address the challenges associated with sustainable alternative feed ingredients in poultry feeding have been made, albeit on a limited scale. While these interventions can benefit small-to-medium scale producers, they have yet to significantly elevate the importance of these ingredients. Nonetheless, several proven procedures exist that have been effective with conventional ingredients and are likely to be effective with alternative ones as well.

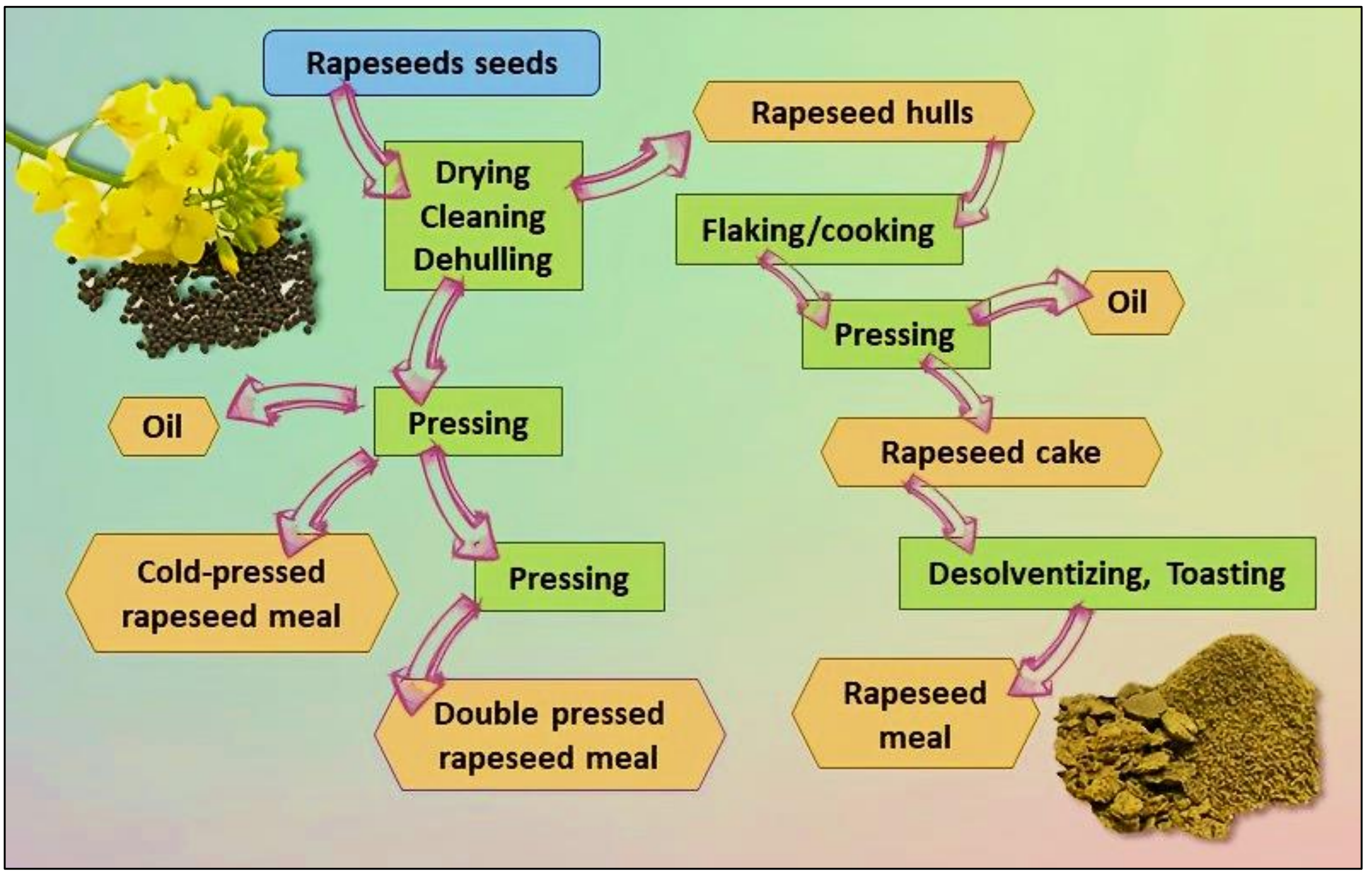
7.1 Crop Breeding

Recent research has focused on enhancing the nutritive value of alternative leguminous crops through crop breeding. The primary goal of this approach focuses on the identification of the various desirable and undesirable characteristics that include the ANFs. Traditional plant breeding is done through genetic manipulation, that is gene transfer, gene silencing, and gene mutagenesis to achieve the aim. Nonetheless, the process is quite slow and results may differ, as seen in the decreased levels of trypsin inhibitors in peas (*Pisum sativum*) [19]. However, when developing crops, it is vital not to eliminate ANFs since they are involved in the plant's defence mechanisms against pests and microorganisms. As a result, the elimination of ANFs before the establishment of their ingestion is a more reasonable solution [153]. In brief, by ensuring that such a crop does not have features considered undesirable for consumption, crop breeding research aims to enhance the nutritional value of a crop.

7.2 Processing

Different strategies can be applied to reduce ANF levels in the feed ingredients as these compounds may vary from type of plant or seed and certain strategies could be applied [154]. The mechanized procedure of dehulling involves the removal of the seed coat or husk which tends to accumulate tannins in many other protein substitutes. Dehulling has been successful in minimizing tannin concentration and this is throughout different seeds from leguminous plants [155-156]. Another common treatment applied in processing legume grains is heat treatment which includes steam heating or toasting that can enhance effective deactivation of ANFs thus improving the nutritional values [157]. Extrusion, which utilizes heat and pressure, proved to have great potential in the decrease of ANF contents in feeds that have been established by some research on Faba beans [158-159]. Processing of legume seeds particularly by cooking also inactivates various ANFs as was noted in the effects of cooking bean seeds in vitro [160]. In draining pulses concentration of α-galactoside can be considerably reduced by germinating, and improvements can be expected when using additives or changing parameters of soaking overnight [161]. Additionally, germination and solvent extraction have been explored for their effectiveness in reducing ANFs and enhancing the nutritive value of alternative protein sources [162]. These methods offer valuable strategies to improve the quality of alternative protein sources for poultry nutrition [19]. The processing of rapeseed begins with the rapeseed seeds undergoing drying, cleaning, and dehulling. From this initial stage, rapeseed hulls are separated as a byproduct. The cleaned seeds then proceed to flaking and cooking, followed by pressing. This pressing stage yields two products: oil and rapeseed cake. The rapeseed cake can undergo a secondary pressing, producing additional oil and double-pressed rapeseed meal. Alternatively, the rapeseed cake can be processed through desolventizing and toasting to produce rapeseed meal. Another pathway involves producing cold-pressed rapeseed meal directly from the seeds. The flowchart thus outlines multiple pathways leading to the extraction of oil and different forms of rapeseed meal from the initial rapeseed seeds (Figure 2). In brief, ANFs in protein sources may be reduced by a variety of techniques, including germination, solvent extraction, heat treatment, extrusion, boiling, soaking pulses, and dehulling. These methods enhance the nutritional value of substitute protein sources for the diet of poultry.

Figure 2: The processing stages of rapeseed into oil and meal products



7.3 Nutrient supplementation

The goal of feed formulation is to create a balanced diet that supplies poultry with the necessary biologically available nutrients. This includes energy, protein, minerals, vitamins, and specific amino acids. When using alternative protein sources, which may lack certain nutrients and contain high levels of ANFs, supplementation becomes essential. Studies have shown significant improvements in diet nutritive value through supplementation with alternative protein sources. For instance, combining cottonseed meal with lysine-rich supplements can replace a considerable portion of soybean meal protein in broiler diets without adverse effects. Similarly, as with any plant product, the gossypol toxicity in cottonseed meal, where it is utilized, can be decreased with iron salts [35]. At the same time, since linatine hinders the growth of poultry, linseed meals can be used as a substitute for other protein feed resources in limited to 10% portions, provided that vitamin B6 as a specific antagonist of linatine is administered as well. Researchers have also recommended amino acid supplementation to eradicate deficiencies associated with other possible substitutes like groundnut cake, cottonseed meal, and linseed [147, 163-164]. In brief, using the formulated feeds from feed milling industries, to provide energy, protein, minerals, vitamins, and amino acids in a ratio to meet the dietary requirement is the aim of feeding. It is always corroborated that when other sources of protein such as the cottonseed meal are incorporated, the diets can always be made richer.

7.4 Non-nutrient supplementation

Supplementation of feed enzymes in diets to enhance nutrient digestibility as a part of the dietary improvement of poultry during the past three decades is another significant progress in the feed Industry. These enzymes are incorporated into diets to assist in the reduction of anti-nutrients, including non-starch polysaccharides and phytate. The addition of feed enzymes in diets helps in improving amino acid profiles and the ME of fed ration. Feed enzyme efficiency differs due to factors such as diet type, source of the enzymes, and supplementation rate [42, 165]. It may indicate that there is better activity of proteolytic enzymes or fewer amino acids are leaked into the bloodstream from the tissues when they are digested. Previous studies have demonstrated that the addition of xylanase and phytase individually or in combination could increase the aerial digestion of proteins thus suggesting anti-and nutritional factors on protein digestion and a consequent endogenous loss of amino acids [39]. In brief, enzymes are added to diets for the highest levels of energy and nutrient content. Therefore, based on the type of meal prepared and whether enzymes are present the presence of anti-nutrients such as phytate and non-starch polysaccharides are decreased thus increasing protein digestibility and decreasing the level of amino acid degradation

8. Impact of Anti-Nutritional Factors On Production and Productivities and Treatment Methods

Anti-nutritional factors (ANFs) negatively impact animal growth, health, and general welfare by disrupting nutrient digestion, absorption, and availability in the feedstuffs [9]. Despite rapeseed meal's nutritional similarity to soybean meal, its practical use is limited due to elevated levels of harmful substances and numerous ANFs like glucosinolates, sinapine, tannins, phytic acid, and crude fibre [9]. Glucosinolates and sinapine are particularly problematic, existing mainly as potassium salts and exerting significant effects on rapeseed application [60, 166]. Glucosinolates, known as goitrogens, are a distinct class of compounds found exclusively in plants, predominantly in dicotyledonous families [56]. Hydrolysis of glucosinolates yields harmful by-products such as oxazolidine thione, thiocyanate, isothiocyanate, and nitriles, which are more toxic than intact glucosinolates [9]. Glucosinolates in rape seed cake may exceed international standards, reaching up to 200 mg/gm [56]. Rapeseed meals can be treated for ANFs using physical, chemical, biological, and crop breeding methods [56, 167]. Physical methods involve myrosinase inactivation and shelling, with steam heating, roasting, microwave treatment, and bulking being common techniques. Milling with water can activate myrosinase to reduce glucosinolate levels while drying enhances volatilization of isothiocyanates [9].

The simplest detoxification method is water immersion, reducing glucosinolates by 98% with a 15-20-minute treatment. Chemical methods include acid and alkaline degradation, metal salt treatment, and solvent extraction, albeit with challenges like heat requirements, cost, and pollution. Solvent extraction using ethanol, carbinol, acetone, or water decreases lower molecular weight glucosinolates. Biological methods involve fermentation and enzymatic hydrolysis to reduce toxins [9]. Crop breeding has successfully developed low-erucic acid and low-glucosinolate varieties, enabling rapeseed products' inclusion in poultry rations at up to 25% without adverse effects [60]. Processing conditions, especially heating, influence seed meal quality by ensuring enzyme inactivation but excessive heating can degrade glucosinolates, decrease available lysine, and reduce protein utilization in monogastric diets [168]. In brief, like soybean meal, rapeseed meal has little practical value since it contains significant quantities of toxic compounds and anti-nutritional factors, such as sinapine and glucosinolates. Physical, chemical, biological, and crop breeding are some of the treatment modalities. On the other hand, overheating in monogastric diets might break down glucosinolates and reduce protein utilisation.

9. Conclusion and Recommendations for Future Studies

Rapeseed contains a lot of protein and has been used as a feed with high nutritional value for poultry. Since meat and bone meals comprise high protein levels, they can be incorporated in compound feed perhaps reducing the need for other protein-based products that are unfriendly to the environment. Rapeseed meal processing includes mixtures, unconsciousness, and fractionation steps, this gives an ideal protein fraction. Aids in increasing the efficiency of processing thus increasing the likelihood of preserving the most indispensable nutrients of rapeseed meal that is used in animal feed hence increasing sustainability. Some of the ways are physical techniques and technological processes like myrosinase inactivation through heat myrosinase inactivation through steaming or roasting milling with water and drying of rapeseed meal. There are also various chemical methods for ANF reduction and these start with acid and alkaline degradation, solvent extraction, supplementation with enzymes, and seed germination respectively. It is also worth noting that crop breeding could be employed to develop low-erucic acid rapeseed that would reduce the levels of ANF in the rapeseed meal and hence improve its/feed value in the feeds for poultry and other animal species. Thus, a combination of these approaches can be helpful for the successful enhancement of the nutritional value and quality of rapeseed meals that can be utilised by the poultry industry as one of the plant protein sources.

Moreover, rapeseed meal contains all or most of the essential amino acids for the formulation of poultry diets. When substituting the poultry diet for rapeseed meal the advantage of lean proteins as well as the proper amino acid profile can be observed and it is true that it can reduce the dependence upon supplements while aiding the growth of the poultry with feedstuff in a more efficient manner. All the enzyme complexes in the poultry feed with rapeseed meal enhance energy realization and nutrient absorption through increased digestibility, reduction of anti-nutritional factors, breaking down of starch for energy value, better absorption of amino acids, and proper secretion for microbial formation in the gut. These enzymes are of very big importance due to their functions of improving the nutritional value of rapeseed meals, nutrient digestion, and utilization, and improving the efficacy of poultry production. In the case of poultry feed, the rapeseed produced in Pakistan is still cheaper as compared to the imported soybean meal thus reducing dependency on imports and increasing the cost of raising poultry. This means we get it from availability, we can get it affordably, and nutritionally it is a good source of protein. So, the nutritional value of rapeseed meal and its environmental sustainability improves further by using sustainable processing techniques like fermentation, irradiation, and autoclaving.

The prospects of the use of rapeseed meal as a source of plant protein for poultry in Pakistan should include the issues of the best approaches to enhance rapeseed meal's processing, the use of enzymes to improve the rapeseed meal's digestibility, the evaluation of the positive effects of the use of rapeseed meal on the environment, the development of poultry diets formulated with rapeseed meal, and the examination of the possible substitutes New practices also require routine efficiency studies and daily monitoring of poultry feed market pricing to understand the cost benefits between rapeseed meal inclusion in poultry diets and soybean meal-based diets and poultry producer costs in the market. By implementing these recommendations, it should be possible to maximize the use of rapeseed meal in preserving the environment, economically sustaining itself, and enhancing poultry productivity in Pakistan.

Authors Contribution

Rohban Hameed: Original draft writeup, **Muhammad Tahir:** Conceptualization & Methodology, **Hammadullah:** writeup and methodology, **Rahat Shah:** Reviewing and Editing **Aamir Khan:** Data Collection, Reviewing and Editing, **Abdullah Iqbal**: Original draft write-up, **Rahat Shah**: Writing, Reviewing & Editing, **Fath Ullah**: Writing and data collection, **Saba Kousar**: Investigation & Reviewing, **Muhammad Imran Khan:** Investigation and editing **Javed Iqbal**: Investigation & Reviewing, **Muhammad Idrees Khan:** Investigation and reviewing

Conflict of Interest

The authors declare no conflict of interest.

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