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INFLUENCE OF VARIOUS METHODS OF PROCESSING SOYBEANS ON PROTEIN DIGESTIBILITY AND REDUCTION OF NITROGEN DEPOSITS IN THE NATURAL ENVIRONMENT – A REVIEW*

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Abstract

Soybean meal (SBM) is a significant source of protein for poultry due to its high concentration of protein and amino acids profile. The primary objective of SBM processing is the reduction and deactivation of anti-nutritional factors (ANFs) to enhance nutrient digestibility. However, eliminating ANFs does not necessarily correlate with increased protein and amino acid content. Several processing methods, such as soaking, cooking, and commercial techniques, improve SBM protein digestibility. To sustain feeding qualities, soybeans must undergo heating to remove inherent chemicals that interfere with poultry digestion. However, both under and over-processing can impair meal digestibility. Extrusion heating significantly contributes to protein and starch digestibility while reducing certain ANFs. Thermomechanical and enzyme-assisted procedures reduce heat-resistant proteins more efficiently than extraction methods, producing soy protein concentrate (SPC) and fermentation. These techniques reduce ANFs in SBM, activating digestive enzymes and alleviating digestive tract pressure, leading to decreased endogenous protein deficits. Exogenous enzyme supplementation is a biotechnological approach for enhancing the nutritional content of SBM and certain other protein-rich products. However, different processing methods not only affect protein digestibility but also impact poultry production, gastrointestinal health, and the environment due to higher nitrogen excrement. Ammonia (NH_3) emissions are a significant environmental concern in chicken farming, resulting from uric acid production during protein consumption. It has a negative impact on the environment and the health of birds/humans. This review investigates the impact of several processing techniques on the digestibility of SBM protein and the reduction of N_2 regarding one health concept. Future research should focus on identifying optimal processing methods maximizing protein digestibility while minimizing environmental nitrogen impacts.

Key words: nitrogen emission, one health, protein digestibility, soybean processing methods

Soybean (*Glycine max* (L.) Merrill) is a leguminous plant grown primarily for its high protein (40%) and fat (20%) content (de Visser et al., 2014). It is commonly used in the feed industry as a protein-rich, defatted, and toasted SBM for livestock (Stein et al., 2008). The rising demand for imported soybean is ascribed to its substantial crude protein (CP) level and amino acid (AA) composition, which is supplementary to cereals, as well as its high AA bioavailability (de Visser et al., 2014; Ravindran et al., 2014). In the European Union (EU), most of the SBM is imported from genetically modified cultivars, and accounts for 80% of plant protein-rich feed materials, and 95% of that was used as livestock feed (Guo et al., 2022; Lannuzel et al., 2022). Soybean agriculture accounts for about 2% of farmlands in the EU, even though this percentage is predicted to rise (EU agricultural outlook for markets, income and environment, 2021–2031) (FAO, 2023).

Genetically modified (GM) soybeans have been subjected to criticism and concerns mostly related to environmental, health, and socio-economic factors. Firstly, a significant concern arises over the environmental consequences associated with the utilization of herbicides, glyphosate (which has been accused of the spread of cancer in humans), which can result in the emergence of herbicide-resistant weeds and pose a possible threat to biodiversity. Secondly, the phenomenon of gene flow, in which genetically engineered features might disseminate to non-genetically modified crops, gives rise to ecological uncertainties. Thirdly, the health issues associated with GM soybeans mostly pertain to the possible presence of allergens, changes in nutritional composition, and the existence of antibiotic resistance indicators. The consolidation of GM seed production among a small number of biotechnology corporations presents difficulties in maintaining the agricultural variety and farmer independence.

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The aforementioned shared concerns highlight the necessity of acquiring a comprehensive comprehension of the possible hazards linked to GM soybeans, with a focus on the significance of responsible and sustainable agriculture methods (de Visser et al., 2014; Sieradzki et al., 2021). European governments have pushed an expansion of local cultivation of non-genetically modified (non-GM) soybeans and other protein-delivering legumes. This progressive approach aims to reduce their reliance on imported protein supplements and mitigate environmental difficulties associated with imported GM soybeans (Martin, 2015; Balázs et al., 2021). In addition to increasing soy farming, a suitable processing framework should be built, as big, optimized processing factories are currently positioned distant from all growing locations. Local small-scale processing may have the ability to increase farmers' protein self-sufficiency. It is generally understood that optimal soybean processing is critical for delivering adequate feeding value. Soybeans have emerged as a well-established staple crop in Austria, followed by maize, wheat, and barley (Carr et al., 2020). In Poland, imported GM soybean meal covered 65%, while locally cultivated leguminous seeds covered 11% of protein requirements for livestock nutrition (Niwińska et al., 2020; Dzwonkowski et al., 2021). Because of its nutritive value, high concentration of crude protein (44–48.5%), and stable amino acid profile, this plant is a suitable protein source for poultry (Dale, 1994). Over half of the soybean meal (SBM) produced in the USA is fed to broiler chickens (Loeffler et al., 2013). Feed makes up 60–70% of the costs of poultry production, with energy being the most expensive component in the feed (Flachowsky and Meyer, 2015; Ahiwe et al., 2018; Noblet et al., 2022). In order to acquire maximum benefits from poultry production (eggs and meat), formulating a diet tailored to meet the nutritional demands of the birds is essential, contributing to the overall economy (Asghar et al., 2021; Sierżant et al., 2023).

The purpose of this updated review is to offer a comprehensive analysis of the changing patterns in the processing of SBM, with a focus on its historical development and recent progress. This improved narrative will provide a more in-depth examination of how these patterns have significantly influenced the enhancement of nutritional value, in terms of protein digestibility and the reduction of ANFs. Producing highly digestible feed also helps to reduce environmental pollution caused by excessive nitrogen outflows. Another strategy for minimizing ammonia emissions from poultry farming is reducing feed protein and supplementing it with indispensable amino acids. These approaches have the potential to lead to positive long-term changes, promoting environmental safety, animal welfare, and human health.

This review evaluates the impact of different processing techniques on SBM protein digestibility and the reduction of nitrogen excrements for poultry production and environmental sustainability.

Material and methods

A systematic review of peer-reviewed articles published in PubMed (www.ncbi.nlm.nih.gov; last accessed on August 2023); ISI Web of Science (www.webofknowledge.com; last accessed on August 2023), Google Scholar (scholar.google.com; last accessed on August 2023), and ScienceDirect (www.sciencedirect.com; last accessed on August 2023) databases was performed covering last 30 years, including older, primary, and original references. More than 60% of the references were from the past 10 years. Studies were selected if they reported different soybean processing methods to remove ANFs and their impact on protein digestibility. A total of 120 studies from 76 different journals and books, and other scientific reports met the specific standards and were included in the present review. The review is divided into the following sections: a description of various ANFs present in soybeans, methods to remove ANFs from soybeans, and their future perspectives in poultry nutrition.

Anti-nutritional factors (ANF) in soybean

The quality of SBM is ascertained by its amino acids concentrations and the presence of chemical components, known as anti-nutritional factors (ANFs). Unprocessed SBM contains ANFs that possess the potential to significantly reduce its nutritional value, resulting in decreased animal health and productivity (Fekadu Gemede, 2014; Rocha et al., 2014). ANFs have a detrimental effect on feed consumption and nutrient digestibility of SBM, which is widely regarded as the primary protein supplement in broiler diets across the world (de Coca-Sinova et al., 2008). These ANFs can negatively impact the digestion of energy, amino acids, and other nutrients, resulting in reduced animal performance, including poultry. Soybeans must be treated to remove these ANFs, which is especially important in the case of monogastric diets. Numerous protease inhibitors known as trypsin inhibitors (Rada et al., 2017), hemagglutinins or lectins (Ebere, 2016; Udeogu and Awuchi, 2016), goitrogens (Barros Dourado et al., 2011; Bajaj et al., 2016), saponins (Omizu et al., 2011; Chaturvedi et al., 2012), and urease are among the harmful components in soybeans (Khan et al., 2013; Real-Guerra et al., 2013). The prevalence of these components in relatively high concentrations limits the use of soybeans and their derivatives in monogastric diets. Those anti-nutritional substances are the main determinants forcing proper processing of soybeans. ANFs, such as trypsin inhibitors, in raw soybeans exert deleterious effects in birds disturbing the digestion process in the gastrointestinal system. Moreover, the nutritional benefit of SBM for poultry is restricted by a number of ANFs that conflict with feed consumption and nutrient utilization. A high concentration of protease inhibitors, particularly trypsin inhibitors (TI), reduces protein digestibility and AA bioavailability. The TI's deleterious effects on monogastric animals are widely documented in the literature (Ravindran et al., 2014, 2017; Dzwonkowski et al., 2021). TI

have been shown to interact with protein metabolism in the gastrointestinal system of young chickens (Goebel and Stein, 2011), and uncooked soybean meal induces pancreas expansion in growing chickens due to the presence of destructive elements in substantial quantities (Dei, 2011; Erdaw and Beyene, 2018; Beukovic et al., 2012; Foley et al., 2013). Furthermore, excessive levels of protease inhibitors (PI) in the diet produce pancreatic hypertrophy/hyperplasia, resulting in poor growth parameters (Jahanian and Rasouli, 2016; Gilani et al., 2012). Pancreatic enlargement is caused by pancreatic secretory stimulation when the pancreas adjusts for the increased number of blockers in the intestinal system. The impacts of raw SBM on chicken development suppression could not be addressed by enriching the diet with methionine, lysine, and so forth (Loeffler et al., 2013).

Methods to remove ANFs in soybean

The soybean is a widely used protein source in poultry feed due to its high protein content but it contains certain ANFs that limit its nutritional value. However, the protein in soybeans is not easily digestible by poultry, which can lead to reduced growth and feed efficiency. To counteract this, various processing methods, including heat treatment, fermentation, enzyme treatment, and chemical treatment methods have been developed to effectively reduce or eliminate ANFs present in soybeans, as shown in Figure 1. By exploring these strategies, we aim to shed light on how they contribute to enhancing the digestibility and nutritional value of soybeans, thus mitigating their adverse effects on poultry performance.

Heat treatment (HT), including roasting or boiling, has been demonstrated to effectively reduce levels of TI and lectins in soybeans (Kumar et al., 2022). This method contributes to enhancing the nutritional profile of soybeans, promoting better digestibility and utilization. Fermentation, used in the production of traditional soy products like soy sauce and miso, has been shown to reduce ANF levels and improve the digestibility of soybeans (Lu et al., 2022). Enzyme treatment, including protease and phytase supplementation, effectively reduce ANF levels and improves the nutritional value of soy products (Samtiya et al., 2021). Another effective method for eliminating ANFs from soybeans is the chemical treatment approach. It has been reported that most of the plant protein contains intramolecular disulfides, if this concentration of disulfide can be decreased then the deleterious effect of ANFs can also be controlled. This strategic approach aims to enhance the overall quality of plant proteins (Xiong and Guo, 2021).

Heat processing to remove ANFs in soybean

Heat processing, including cooking, roasting, toasting, and extrusion, is one of the most common methods used to improve soybean protein digestibility. This method denatures the proteins and reduces the activity of ANFs, such as TI, lectins, and protease inhibitors (Figure 2). In addition, heat processing enhances the availability of essential amino acids, making the protein more easily digestible for birds. Several studies which show the effect of heat processing methods on soybean digestibility are presented in Table 1.

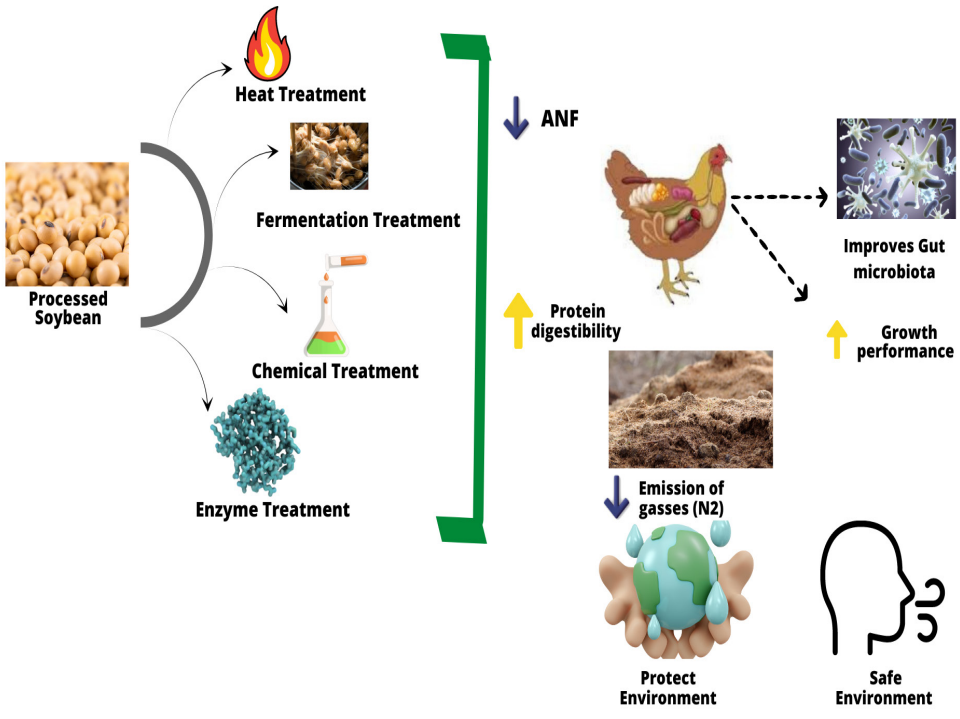


Figure 1. Schematic representation of various processing methods of soybean with respect to protein digestibility and their effect on the environment (own elaboration)

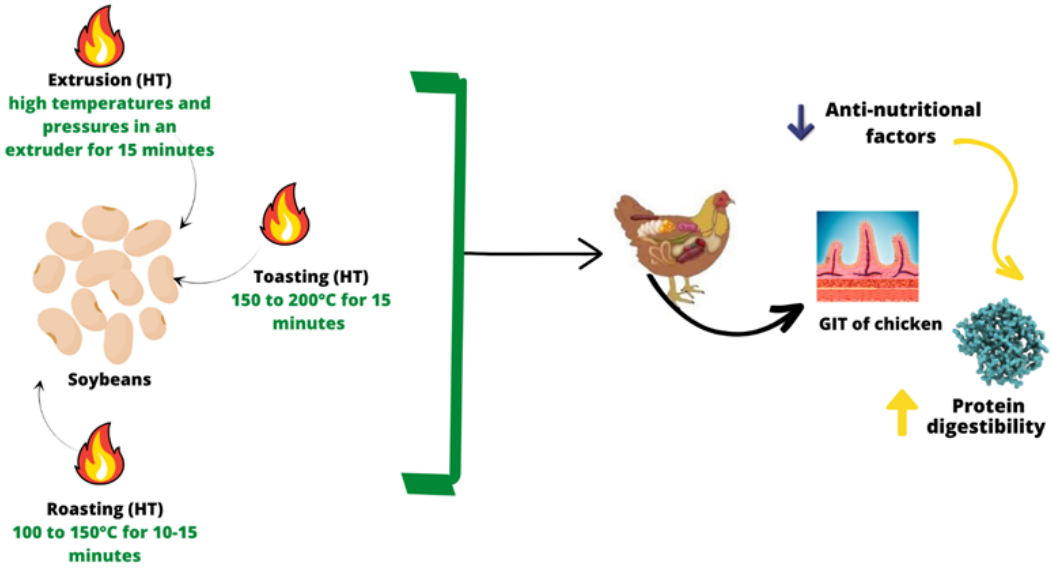


Figure 2. Schematic representation of heat processing methods on soybean and their effect on protein digestibility in poultry (own elaboration)

Table 1. Effect of heat processing method on digestibility of soybean protein and on ANF

References	Species	Diet	Processing method	Processing parameters	Effect
1	2	3	4	5	6
Abdollahi et al., 2022 a	broilers	full fat soybean meal	Wet heating (WH), expansion temperature (EXT)	WH at 80°C for 1 minute, EXT at 115–125ºC	AME, and SID AA lowest
				WH at 100ºC for 6 minutes, EXT at 115–125ºC	AME, and SID AA intermediate
				WH at 100ºC for 16 minutes, EXT at 115–125ºC	AME, and SID AA highest, reduced TIA
Clarke and Wiseman, 2007	broilers	full fat soybean meal	Extrusion temperature (ET)	Extrusion at 90, 110, 130 and 160ºC	Reduced TIA from 28 mg/g to 1.8 mg/g, highest digestibility of AA
Abdollahi et al., 2022 b	broilers	full fat soybean meal	Conditioning and expansion	Conditioning at 100ºC for 6 minutes and expansion with 18 KWh/t	AME and SID of AA ↑, Inhibit or ↓ TIA, KOH solubility
				Conditioning at 100ºC for 9 minutes and expansion with 28 KWh/t	ns effect on AME and SID of AA, TIA (3.9 mg/g KOH solubility)
Leeson and Atteh, 1996	broilers	full fat soybean meal	Extrusion temperature (ET)	Extrusion at 80, 100, 120 and 140ºC	TIA reduced 16.1, 14.8, 9.3, and 8.4 mg/g respectively
Oliveira et al., 2005	broilers	full fat soybean meal	Extrusion temperature (ET)	Extrusion at 125, 130, 135 and 140ºC	ns effect on carcass composition and performance
Herkelman et al., 1991	broilers	full fat soybean meal	Extrusion temperature (ET)	Extrusion at 121ºC for 40 minutes	significant effect on carcass composition and performance
Perilla et al., 1997	broilers	full fat soybean meal	Wet extrusion temperature (WET)	Optimum WET at 122 and 126ºC	ns effect on carcass composition and performance
Sakkas et al., 2019	broilers	soybean meal	Extrusion-pressing (E)	140ºC at around 100 kg/h	ns effect on AME and SID of AA, TIA (2.6 TIU/mg) ↓
			Flaking-pressing-cooking (F)	After flaking cooked at 150ºC for 3600 s	ns effect on AME and SID of AA, TIA (3.6 TIU/mg)

Table 1 – contd.

1	2	3	4	5	6
Heger et al., 2016	broilers	full fat soybean meal	Conditioning and expansion	Expanding at 125ºC for 15 s, short-term (1 minute, 100ºC) and long-term (5 minutes, 100ºC) conditioning	TIA, urease activity, and PDI values ↓, solubility of protein unchanged
Ari et al., 2017	broilers	soybean meal	Extrusion temperature (ET)	Extrusion temperature (ET)	Performance trait score 2, nutrient digestibility of CP 83.03, reduced TIA 60.59%,
			Cooking	Cooking	Performance trait score 3, best performance, ANF ↓, digestibility of AA↑, nutrient quality↑, nutrient digestibility of CP 83.62, reduced TIA 85.02%
			Toasting	Toasting	Performance trait score 1, nutrient digestibility of CP 81.86, reduced TIA 52.44%
			Roasting + dry heating	Roasting + dry heating	Performance trait score 4, nutrient digestibility of CP 81.72, reduced TIA 53.75%

AME: Apparent metabolizable energy; AID: Apparent ileal digestibility; SID: Standardized ileal digestibility; CP: Crude protein; AA: Amino acids; TIA: Trypsin inhibitor activity; ↓: Decrease; ↑: Increase; –: No changes. Ns: No significant; PDI: Protein dispersion solubility index; EXT: Expansion temperature; ET: Extrusion temperature.

SBM is a popular protein source used in livestock diets, however, its quality can be diminished through excessive heat processing. Processing technologies, such as heat treatment (cooking, toasting, roasting, and extrusion-dry heating), can reduce the presence of ANFs in this raw material (soybeans). On the other hand, this process can also reduce the digestibility and availability of essential amino acids, such as lysine, arginine, and tryptophan. As a result, optimal heat processes and processing conditions are required for sufficient thermal decomposition of TI while still preserving the nutritional content of SBM (Abdollahi et al., 2022 a). Several trials were conducted to determine the ideal processing conditions for feeding acceptability (Abdollahi et al., 2022 b; Kuenz et al., 2022; Clarke and Wiseman, 2005). To optimize animal performance, reducing soybean TI activity (TIA) to 3–4 g/kg can be achieved through appropriate processing techniques such as HT or enzymatic methods. Nevertheless, certain independent investigations have revealed that TIA levels beyond the recommended range may be tolerable or even preferable, highlighting the fact that soybean feeding acceptability is influenced by factors other than TI (Heger et al., 2016; Hoffmann et al., 2019; Sakkas et al., 2019).

Heat treatment (HT), such as extrusion cooking, has been found to increase the digestibility of soybean protein in poultry by denaturing the proteins and making them more accessible to digestive enzymes. There are several HT methods used in the processing of SBM, including roasting, toasting, and extrusion-dry heating. Roasting (dry heating) involves heating soybeans at temperatures of 100 to 150°C for 30 minutes, while toasting involves heating soybeans at temperatures of 150 to 200°C for shorter time 30 minutes. Extrusion involves subjecting soybeans to high temperatures (140°C) for 8 minutes, and pressures in an extruder, resulting in the denaturation of ANFs and improved digestibility of the protein (Rostagno et al., 2011; Ari et al., 2013 a, 2017; Hemetsberger et al., 2022). Several studies have demonstrated the effectiveness of HT in reducing ANFs content in SBM. For example, a study by Rostagno et al. (2011) showed that roasting at 120°C for 90 minutes reduced TIA by 97%, while another study by Hemetsberger et al. (2022) found that extrusion at 140°C for 8 minutes reduced the content of ANFs such as lectins and phytic acid. Extrusion (Clarke and Wiseman, 2007), toasting over sustained fire (Herman et al., 2011), hot air, or infrared radiation (Wiriyaumpaiwong et al., 2004) are among the ways used to reduce TIA. Typically, soybeans are heated following a solvent extraction stage to isolate the soybean oil (Gerde et al., 2020). Direct and indirect steam warms the extracted commodities to 50–60°C for 90 minutes during solvent extraction in hexane (Newkirk et al., 2003). The effectiveness of HT method is dependent on the specific HT process used, as well as the temperature, duration, and pressure used in the treatment. These factors should be carefully considered to optimize the nutritional value of SBM for animal diets (Colombino et al., 2023). However, treatment processes will have a significant influence on the nutritional value of soy products in addition to the general digestibility of the meal. Although heat is a necessary stage in the processing of soybeans, extreme HT might reduce nutritional digestibility, particularly for protein, due to the development of potentially hazardous compounds such as Maillard product lines (Ton Nu et al., 2020). Overheating promotes Maillard activities between the amino group of the AA and reducing sugars (such as glucose and galactose), lowering energy and AA bioavailability (Sajid et al., 2023; Lee and Stein, 2022).

Table 2. Effect of fermentation processing method on digestibility of soybean protein and on ANF

References	Fermenting agent	Substrate	Protein digestibility changes compared with raw substrate	ANF changes compared with raw substrate	AA and protein digestibility changes compared with control
1	2	3	4	5	6
Cervantes-Pahm and Stein, 2010	*Aspergillus oryzae*	SBM	CP ↑, AAs ↑	Trypsin inhibitor activity, stachyose ↓, raffinose	AID-CP –, SID-CP –, AID-LYS ↓, AID and SID of the rest of AA –
Opazo et al., 2012	*B. subtilis, S. thermophilus*, *Saccharomyces cerevisiae*	SBM	CP ↑	Total NSPs ↓, stachyose ↓, raffinose ↓	
Rojas and Stein, 2013	*Aspergillus oryzae, Bacillus subtilis*	SBM	CP ↑, AAs ↑	Glycinin ↓, β-conglycinin ↓	SID-CP –, SID-Met ↑, SID-Val ↑, SID-rest of AAs –
Upadhaya and Kim, 2015	Yeast and *Bacillus strains*	SBM	CP ↑, AAs ↑	β-conglycinin ↓, trypsin inhibitor activity ↓, stachyose and raffinose ↓	AID-CP ↑, SID-CP ↑, AID-AAs –, SID-AAs –
Jeong et al., 2016	*Enterococcus faecium*	SBM	CP ↑, Sulphur containing AAs ↓	Trypsin inhibitor activity ↓, stachyose ↓, raffinose ↓	AID-CP ↑, AID-Arg ↑, AID-Lys ↑, AID-Asp ↑, AID-Gly ↑, AID-Glu ↑, AID-Tyr ↑, AID-essential Aas ↑
Shi et al., 2017 a	*Bacillus subtilis, Enterococcus faecium*	SBM, corn	CP ↑, Sulphur containing AAs ↓	Trypsin inhibitor activity ↓, glycinin ↓, β-conglycinin ↓	AID-CP –
Jazi et al., 2019	*Lactobacillus acidophilus, Lactobacillus plantarum, Bacillus subtilis, Aspergillus oryzae*	SBM	CP ↑, AAs ↑	Glycinin ↓, β-conglycinin ↓	
Ari et al., 2017	Fermentation with culture organisms *Lactobacillus*	SBM	Performance trait score 4, nutrient digestibility of CP 85.91, reduced TIA 100%	Reduced TIA 100%	
	Cooking and fermentation	SBM	Performance trait score 3, best performance, ANF ↓, digestibility of AA ↑, nutrient digestibility of CP 87.07, reduced TIA 100%	Reduced TIA 100%	
	Daddawa fermentation	SBM	Performance trait score 1, nutrient digestibility of CP 86.55, reduced TIA 100%,	Reduced TIA 100%	
	Cooking and ferment + potash fermentation	SBM	Performance trait score 2, nutrient digestibility of CP 85.91, reduced TIA 100%	Reduced TIA 100%	
Li et al., 2020	*B. amyloliquefaciens, L. acidophilus, Saccharomyces cerevisiae*	SBM	Growth performance ↑, nutrient digestibility ↑, serum immunity ↑	ANF ↓	Digestibility of nutrients ↑

Table 2 – contd

1	2	3	4	5	6
Soumeh et al., 2019	*Lactobacillus acidophilus, Lactobacillus plantarum, Bacillus subtilis, Aspergillus oryzae*, Mannan-oligosaccharide (MOS)	corn, SBM	Gut morphology improved ↑, nutrient digestibility ↑, serum immunity ↑	ANF ↓, TIA ↓	Intestinal activity of amylase, protease, and pancreatic protease ↑, AID of CP ↑
Jazi et al., 2019	*Lactobacillus acidophilus, Lactobacillus plantarum, Bacillus subtilis, Aspergillus oryzae*	SBM	Intestinal morphology improved ↑, nutrient digestibility ↑	ANF ↓, TIA ↓	AID and AME of CP ↑, digestibility of nutrients ↑, gut pathogenic microbes ↓
Feng et al., 2007	*Aspergillus oryzae* 3.042	SBM	Growth performance ↑, IgA, IgM↑	ANF ↓, TIA ↓	Digestibility of nutrients ↑, IgA ↑, IgM ↑

AME: Apparent metabolizable energy; AID: Apparent ileal digestibility; SID: Standardized ileal digestibility; CP: Crude protein; PDI: Protein dispersion solubility index; AA: Amino acids; TIA: Trypsin inhibitor activity; ↓: Decrease; ↑: Increase; –: No changes. Ns: No significant.

Hence, useful markers for assessing the impact of heat processing on the protein quality of soybean products have been explored (Zaheer and Akhtar, 2017). Protein solubility in KOH has been established as a straightforward and low-cost technique for estimating protein breakdown due to over-processing (Araba and Dale, 1990). Meanwhile, additional approaches, including direct amino acid profiling, reactive lysine, and Maillard compounds, were used in the analytical assessment (Rutherfurd and Moughan, 2012).

Fermentation processing method

Fermentation, as a soybean processing method, enhances protein digestibility and reduces the presence of ANFs, including TI and phytates, which may otherwise impede protein digestion. A study by Wang et al. (2009) found that fermentation of soybeans with *Bacillus subtilis* increased the digestibility of soybean protein in broiler chickens by 15%. The fermentation method entails the proliferation of lactic acid bacteria, effectively breaking down lectins, protease inhibitors, and phytic acid. In addition, fermentation can enhance the nutritional value of SBM by lowering the content of TI and increasing the digestibility and bioavailability of AA, minerals, and vitamins (Li et al., 2020; Abdel-Raheem et al., 2023). Moreover, fermented SBM modulates the gut microbiome by producing beneficial (lactic acid-producing) bacteria (LAB), and decreasing the pathogenic bacteria in the gastrointestinal tract (GIT) of birds, thereby functioning like probiotics (Shi et al., 2017 b; Soumeh et al., 2019).

Fermentation additionally reduces the molecular weight of compounds through biochemical changes resulting from this process. Fermentation breaks the complex soybean molecules into simpler ones, increasing the surface area, and concentration of water-soluble components, which increases the protein availability (Kiers et al., 2000). Several studies which show the effect of fermentation processing methods on soybean digestibility are presented in Table 2.

There are two types of fermentation: submerged fermentation (SF) involves the dissociation of nutrients in the presence of plenty of free water, and solid-state fermentation (SSF) involves the dissociation of nutrients in the presence of microbes, without free water (Lonsane et al., 1985; Chahal, 1991). SF involves microorganisms submerged in a liquid medium, providing precise control over moisture, efficient nutrient accessibility, and easier heat dissipation. This method is often favored for large-scale industrial processes, such as pharmaceutical and enzyme production. SSF is the newest form of fermentation, which shows more advantageous effects over SF including low cost of treatment, no problems of foaming, more chances of product recovery, and reduced effluent production. SSF also imitates the natural habitat of microorganisms, moreover, the production of enzymes in this type of fermentation is very high. A few demerits of using this SSF have also been reported which include: more chances of contamination, and sometimes difficulties with controlling moisture and heat levels in some conditions (Lonsane et al., 1985; Abdel-Mageed et al., 2022).

Table 3. Effect of enzymes processing method on digestibility of soybean protein and on ANF

References	Species	Diet	Enzymes	Effect
Cowieson et al., 2020	broilers	corn, SBM	phytase and protease	AID and SID AA ↑, proteinaceous ANF ↓
Aderibigbe et al., 2020	broilers	SBM	monocomponent protease	Improved BWG, FCR, AID AA ↑, trypsin and chymotrypsin activity ↑, villus length and width ↑
Erdaw et al., 2017	broilers	SBM, FFSB	microbial protease	Trypsin and chymotrypsin activity ↑, pancreatic digestive enzymes ↑, AID of AA and CP ↑
Costa et al., 2008	poultry	SBM	exogenous enzymes, protease	Proteinaceous ANF ↓, digestion of nutrients ↑,
Clarke and Wiseman, 2005	broilers	SBM	protease	Proteinaceous ANF ↓, digestion of AA ↑, TIA ↓
Ghazi et al., 2002	broilers	SBM	protease	AID of nitrogen and nitrogen retention ↑, improved chick performance, BWG, FCR
Ghazi et al., 2003	broilers	SBM	protease and α-galactosidase	True metabolizable energy and true nitrogen digestibility ↑, improved chick performance, BWG, FCR
Angel et al., 2011	broilers	SBM	monocomponent protease	AID of methionine ↑, AID of CP ↑, ANF ↓
Bertechini et al., 2009	broilers	SBM	protease	True digestibility of AA ↑,
Cowieson et al., 2013	poultry	SBM	protease	ns effect on methionine digestibility digestibility of AA ↑
Kocher et al., 2003	broilers	corn, SBM	pectinase, protease and amylase	AME and AID of AA ↑, ANF ↓
Camden et al., 2016	broilers	corn, SBM	microbial phytase	AME and AID of AA ↑, ANF ↓, ileal digestibility of nitrogen, AA, starch and lipids↑
Jiang et al., 2022	broilers	corn, SBM	multienzymes (β-mannanase)	AME of AA ↑, ANF ↓, villus height to crypt depth and starch and lipids↑
Wang et al., 2008	broilers	SBM, cotton seed	keratinase	Growth performance ↑, BWG, starch and nitrogen digestibility ↑, villus height to crypt depth↑

AME: Apparent metabolizable energy; AID: Apparent ileal digestibility; SID: Standardized ileal digestibility; SBM: Soybean meal; CP: Crude protein; BWG: Body weight gain; ANF: Anti-nutritional factors; PDI: Protein dispersion solubility index; AA: Amino acids; TIA: Trypsin inhibitor activity; ↓: Decrease; ↑: Increase; –: No changes. Ns: No significant.

Enzymatic processing method

Enzymatic processing, a well-established method utilized for years, employs enzymes to break down ANFs, thereby enhancing protein digestibility in soybeans. Proteases and phytases play a vital role in breaking down indigestible components of soybeans, specifically targeting substances like TI and phytates, thereby enhancing protein digestibility. A study by Kim et al. (2021) found that enzyme treatment with a combination of protease and phytase increased the digestibility of soybean protein in broiler chickens by 20% and apparent ileal digestibility (AID) of gross energy by 3%. Protease enzymes can hydrolyze protease inhibitors (PI), while phytase enzymes work to degrade phytic acid. This enzymatic action results in the effective reduction of ANFs, contributing to improved protein digestibility. This method can effectively reduce lectins, protease inhibitors, and phytic acid, while also preserving the protein content of soybean. This processing method is particularly useful for the production of high-quality animal feed, as it results in improved production performance of birds. Several studies which show the effect of enzymatic processing methods on soybean digestibility are presented in Table 3.

Modification in protein structure can be done by enzymatic processing methods in two ways: by direct addition of enzymes to the substrate or by medium inoculation with certain types of microbes, which sequentially ferments this medium and produces enzymes (Velázquez-De Lucio et al., 2021). Several different types of enzymes like β-glucosidase, α-galactosidase, phytases, and protease are most frequently used to nullify the effect of ANFs, inhibitors, oligosaccharides, and improve the digestibility of protein, as well as the availability of phosphorus (Cardoso et al., 2020). Enzymatic processing plays a crucial role in

modifying the protein structure of soybeans, thereby enhancing their digestibility and nutritional value. Through specialized methods like the "enzyme-assisted aqueous extraction processing (EAEP) of soybean", developed by Iowa State University, enzymes are strategically applied to induce specific changes in the protein composition. These modifications aim to improve the functionality and bioavailability of soybean proteins, contributing to better overall nutritional outcomes. In addition to this, enzymes can also be used to extract soybean oil by adopting the specialized method EAEP. By-product of this method is soybean cotyledon fiber (Machida et al., 2022).

Chemical processing method

Chemical processing, including acid and alkali treatment, can also improve the digestibility of soybean protein. These methods can break down ANFs, such as phytic acid and oligosaccharides, thereby increasing the bioavailability of essential AA. However, chemical processing can also reduce the protein content of soybeans, so it is important to carefully monitor the processing conditions to ensure that the desired outcomes are achieved. Several studies which show the effect of chemical processing methods on soybean digestibility are presented in Table 4.

The majority of protein in soybean presents in the form of glycinin, which agglomerates with disulfide bonds, and those are mostly present in plant inhibitory proteins (Fu et al., 2021). Two different kinds of plant inhibitory proteins are present in soybean: Kunitz inhibitor (trypsin-inhibitor) and Bowman-Birk inhibitor (chymotrypsin-inhibitor) which mostly prevent the digestion of protein (Rebollo-Hernanz et al., 2023). There are seven intramolecular disulfide bonds present in chymotrypsin inhibitors and only two in TI (Cid-Gallegos et al., 2022). Some ANFs are resistant to heat; however, the denaturation of ANFs of soy protein at high temperatures is limited to a minor extent. Only 80% TI was inactivated by heating at 120°C for 30 minutes (Mittal et al., 2021). There may be a modification in the three-dimensional structure of protein during the chemical processing method because of reduction that makes the protein more prone to the digestion process (Abdollahi et al., 2022 a). It was reported that the digestibility of soybean has been increased by chemical treatment with sodium sulfite and metasulfite even at a mild temperature (Wang et al., 2009). This chemical treatment causes inactivation of TI, and reduction of disulfide bonds, which in turn improve the *in vitro* protein digestibility even at a temperature of 55°C. The digestibility of soy protein was three times higher at 100°C compared to 80°C temperature (Wang et al., 2009).

Table 4. Effect of chemical processing method on digestibility of soybean protein and on ANF

References	Species	Diet	Chemical method	Effect
Ari et al., 2017	broilers	SBM	Soaking in water (HOH) 0% alkaline	Performance trait score 3, nutrient digestibility of CP 80.62, reduced TIA 38.76%,
			Sodium carbonate 1% (Na_2CO_3)	Performance trait score 3, best performance, ANF ↓, digestibility of AA↑, nutrient digestibility of CP 79.26, reduced TIA 94.14%
			Potassium carbonate 1% (K_2CO_3)	Performance trait score 1, best performance, ANF ↓, digestibility of AA↑, nutrient quality improved, nutrient digestibility of CP 80.96, reduced TIA 92.18%
			Sodium hydroxide 1% (NaOH)	Performance trait score 2, nutrient digestibility of CP 75.10, reduced TIA 92.51%
Ari, 2014	broilers	SBM	0% Alkaline	CP 39.70%, Lys 5.23%, TIA 9.40 mg/k, reduction in TIA 38.76%, PA 325%, reduction in PA 5.80%, Ca 0.39%, P 0.36%
			1% Na_2CO_3	CP 38.34%, Lys 5.50%, TIA 0.90 mg/k, reduction in TIA 94.14%, PA 66%, Reduction in PA 80.87%
			1% K_2CO_3	Highest CP 40.27%, Lys 5.78%, TIA 1.20 mg/k, reduction in TIA 92.18%, PA 48.8%, reduction in PA 85.86%
			1% NaOH	Moderate CP 39.64%, Lys 4.79%, TIA 1.15 mg/k, reduction in TIA 92.15%, PA 48.6%, reduction in PA 85.91%
Ayanwale, 2003	broilers	SBM	sodium sesquicarbonate	Toxic polyphenols ↓, TIA ↓, digestibility of AA ↑, energy utilization ↑
Ari et al., 2012	broilers	SBM	alkali treated soybean	Performance traits ↑, nutrient digestibility ↑, economic returns↑
Ayanwale, 2006	broilers	SBM	sodium chloride, sodium sesquicarbonate (trona) and alum	Inactive TIA, polyphenols and oligosaccharides ↓, BWG ↑
Ayanwale, 1999	broilers	SBM	sodium sesquicarbonate	Toxic polyphenols ↓, TIA ↓, digestibility of AA↑, energy utilization ↑, retention of nitrogen ↑
Ari et al., 2013 a	broilers	SBM	sodium carbonate, potassium carbonate, sodium hydroxide	Improved carcass yield in potassium treated soybean, TIA reduced

AME: Apparent metabolizable energy; AID: Apparent ileal digestibility; SID: Standardized ileal digestibility; CP: Crude protein; PDI: Protein dispersion solubility index; AA: Amino acids; TIA: Trypsin Inhibitor activity; ↓: Decrease; ↑: Increase; –: No changes. Ns: No significant; SBM: soybean meal.

Future perspectives

The drive to mitigate the environmental footprint of agriculture necessitates advancements in livestock nutrition. These innovations encompass both the feed product and the feeding approach. Efforts are being made to use high-protein feedstuffs that are sourced locally, thus reducing transportation-related greenhouse gas emissions. This is particularly important within a confined crop-livestock integration system where nutrient cycling is restricted, thus reducing nitrogen runoff. One potential solution is based on the substitution of conventional protein feedstuffs with a reduced crude protein content, augmented with essential amino acids. This aims to diminish ammonia and greenhouse gas emissions, thus abating the environmental impact of agriculture (Chojnacka et al., 2021).

Europe has a societal expectation for the implementation of agricultural practices that will curb climate change, pollution, and enhance the safety of food derived from animal sources. To meet these expectations, in recent years, there has been an increase in the cultivation of non-genetically modified soybeans (*Glycine max* (L.) Merr), facilitated by the European strategy supporting leguminous crop growth. Adaptation of high-yielding seed, protein, and fat varieties to local, European agro-climatic conditions is underway (Sieradzki et al., 2021).

As energy consumption and greenhouse gas production in the livestock sector are anticipated to escalate, it is imperative to leverage technological advancements to support livestock farming, particularly in the domain of technical improvements. There is a need to develop high-protein feed components to ensure protein safety within the EU. The ongoing development of innovative feed aims to enhance digestibility, facilitate efficient fat and protein absorption, and provide resistance to diseases and pests. To bring these innovative feed to market, advances in processing technologies, including optimal time, temperature, pressure, and humidity parameters, must be made. The functionalities and market-attractive parameters of these products must be verified through digestibility and production trials on farm animals (Guo et al., 2022; Abdel-Raheem et al., 2023).

The successful integration of locally cultivated soybean varieties and their processed products into poultry feed depends on the precise selection of feed components and the synchronization of nitrogen and energy release during gastrointestinal fermentation. Improving protein utilization efficiency in monogastric animal nutrition e.g., broiler chickens, is an indicator of the positive impact of sustainable development principles. The nitrogen absorption level from feedstuffs can be estimated through animal studies, by differences in nutrient content between feed and feces calculated in comparison to a control group.

Optimal protein digestibility is essential for poultry production and environmental health. Poultry production is a major contributor to nitrogen excrement and ammonia production, which can have negative impacts on the environment and human health. Therefore, feed processing methods that can reduce nitrogen excrement and ammonia production are of great interest. A review of the literature suggests that soybean crops can be utilized in poultry feed in various forms, including raw seeds and thermally or mechanically processed products, each with unique nutritional properties and limitations. Livestock manure, including litter and liquid manure, supplies large amounts of nitrogen to the soil via unused protein in feed. During the production, storage, and application of natural fertilizers, nitrogen is lost from manure in the form of ammonia and volatile organic compounds, causing odor nuisance from livestock buildings. Thus, any technology that increases nitrogen bioavailability for farm animals is highly advantageous for environmental protection and the sustainable development of the animal food production sector.

Conclusion

In a nutshell, soybeans, though containing ANFs that affect digestibility, can be transformed through various processing methods like heat, chemical, enzymatic, and fermentation. These methods have a substantial effect on protein digestibility, ANFs, and poultry production performance. They provide opportunities to improve growth, feed efficiency, and sustainability in animal production. The selection of a processing method depends on specific outcomes, available facilities, and raw soybean quality. Sustainable agriculture, coupled with economic benefits, emphasizes the importance of improving nutrient efficiency, particularly with nitrogen, to address environmental stress. Utilizing locally sourced, easily digestible protein feed can reduce greenhouse gas emissions and prevent pollution of the environment. Reducing the protein content in poultry feed, coupled with indispensable amino acids after processing, provides a method to decrease ammonia emissions. Adopting these processing techniques has the potential to significantly benefit environmental preservation, animal welfare, and human well-being. It is essential to assess various processing methods for SBM protein digestibility and nitrogen excretion for producing environmentally sustainable poultry feed. Future research should focus on identifying optimal methods to maximize protein digestibility while minimizing negative environmental impacts. This will contribute to enhancing the nutritional quality and reducing the environmental footprint of soy-based poultry feed.

Conflicts of interest

The authors declare no conflict of interest.

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