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Lentil (*Lens culinaris*) and its by-products inclusion in livestock nutrition: present insights and emerging trends in rabbit and poultry system

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ABSTRACT

The current agro-industrial system must develop a more efficient and sustainable food production model to achieve greater food security for the expanding global population, in alignment with the Sustainable Development Goals (SDGs) outlined in the European Agenda 2030. A green and sustainable solution for the feed-food production dilemma may be the use of agricultural by-products (AB) in livestock feeding. The ABs are what remains of fruits and vegetables after industrial processing, containing a plethora of bioactive compounds. Currently, their direct utilization in livestock nutrition is growing, but more research is needed to deeply discover their potential in terms of health-promoting compounds. Lentils (*Lens culinaris* L.), belonging to the Fabaceae family and commonly categorized as pulses, have historically served as a staple food for humans. Post-harvest processing of lentils yields various by-products (e.g. straw, hulls, screenings) that possess high nutritive value and are rich in phytochemicals, making them suitable for incorporation into balanced diets for numerous livestock species, including poultry and rabbits. This review focuses on the available knowledge on lentil by-products (LB), their nutritive value and phytochemicals including the main dietary effects on growth and productive parameters of poultry and rabbits.

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Introduction

Globally, the significant imbalance between food production and consumption is depicting an alarming reality (Ghany et al. 2023). This can be estimated from the fact that the discarded fraction of the fruit and vegetable processing industries represents about 30% of total food production (Laufenberg et al. 2003; Ajila et al. 2012). Global food processing waste-related greenhouse gas emission was found to be the third highest contributor after total emissions for China and the USA (FAO 2013). In developed countries such as Europe, fruit and vegetable by-products were found to be the fifth highest contributor (8% of total food waste) to overall food waste (Fava et al. 2015). On the other hand, limited data from developing countries indicate that large-scale industries process fruit by-products into biogas or compost whereas waste from unorganized processing industries eventually ends up in municipal waste disposal systems (Banerjee et al. 2017).

Agricultural by-products (ABs) comprise all the remaining parts of vegetables and fruit after harvesting, transportation or industrial processing (Ciptaan et al. 2022; Reguengo et al. 2022; Mohamed et al. 2023). Many studies pointed out the huge quantity of bioactive compounds in ABs that are essentially primary and secondary metabolites of plants (Reguengo et al. 2022; Umeghalu et al. 2022). Interestingly, chemical

studies shown that often the concentration of the latter is greater in the by-product compared to the fruit pulp (Guo et al. 2003; Da Silva et al. 2014). This wide range of compounds includes: phenolics, alkaloids, glycosides, mucilage, gums and tannins as some of the secondary metabolites (Biesalski et al. 2009; Degla et al. 2022; Raza et al. 2022). The abundance and variety of natural components in ABs make it worth investigating in terms of their alternative use to reduce the disposal of these valuable feedstocks.

The elaboration of strategies to find alternative paths for ABs could help to assess both the environmental and socio-economic issues of their disposal (Brunetti et al. 2022). Moreover, in an attempt to reach the sustainable development goals of Agenda 2030 (UN 2015), the valorisation of by-products promotes bioeconomy reducing food losses and making the feed production system more resilient and efficient.

The limited use of ABs as a valuable resource can be overcome with the help of academia to enhance awareness about their possible applications. The increasing modern appeal toward green chemistry and eco-friendly processes has attracted interest in working with the food wastes; their use in feeding livestock underlines the existing complementarity between animal production and human food production (Banerjee et al. 2017; Beltranena and Zijlstra 2022).

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Traditionally, ABs have been used as livestock feed; however, globalization and the reliance on more efficient and complete feedstuffs like soybean meal (SBM) (Laudadio and Tufarelli 2011), led to a decreasing trend in using ABs (Rauw et al. 2023). However, AB's inclusion in livestock rations might reduce feeding costs by relying on cheaper, equally nutritious and even locally-produced feedstuffs (Woyengo et al. 2014; Suliman et al. 2019). Moreover, several investigations have assessed their nutritional content and nutraceutical properties, concluding different ABs can be included in poultry diets at inclusion levels up to 60% (Truong et al. 2019).

Global population is expected to reach 9 billion by 2050 (The World Bank 2019), likewise demand for animal products, such as poultry, is also deemed to increase around 60-70% (Makkar et al. 2016). This will probably result in a need for alternative livestock feed such as pulses protein to reduce the use of traditional expensive feeds. Thus there is a growing interest among researchers and feed producers to provide high-quality feed as alternative to common feedstuffs, capable also to cover energy and protein requirements of animals and to promote more sustainable livestock farming systems (Ayaşan et al. 2018; Biesek et al. 2020).

This review intends to be an awareness enhancer about the potential benefits of using ABs such as lentil by-products (LBs) as a cheaper and available livestock feed. Moreover, we highlighted the biological value of ABs' compounds and the environmental advantages of their use, presenting also the current situation and future trends of LBs implementation, with a focus on poultry and rabbit nutrition.

The literature search included lentil's chemical and phytochemical profile, the by-products resulting upon threshing and subsequent procession (LBs) and their use as livestock feed, was carried out through the use of Scopus, PubMed, Google Scholar and other major search engines. Priority was given to recent articles published in the last decades (1990–2024). Prior, highly-cited papers were also included. To carry out literature research the following keywords were used: 'agricultural by-products', 'agricultural waste', 'agricultural and animals', 'legume', 'pulse grains', 'lentil characteristics', 'lentil by-products', 'lentils as animal and/or livestock feed', 'lentil by-products and poultry', 'lentil by-products and rabbits'.

Phytochemicals in agricultural by-products

Many studies have assessed that by-products are a rich natural source of biocompounds exerting a wide range of biological functions (Kumar et al. 2017; Zhong et al. 2018; Hegazy et al. 2023; Arief et al. 2023; Raza et al. 2023; Khan et al. 2023; Raza et al. 2023; Nasir et al. 2023, 2024). This is also confirmed by the vast array of extraction methods used to obtain added-value products with nutraceutical properties from ABs (Galanakakis 2012; Brunetti et al. 2022; Dim et al. 2022). Traditional extraction methods are often associated with environmental downsides such as high solvent and energy consumption as well as the risk of thermal degradation of heat-labile functional components reducing the extract's bioactivity. Nevertheless, alternative, more efficient and greener extraction methods have been developed and associated with reductions in overall extraction cost, solvent-related risk, energetic input

and removal of wastewater post-treatment (Chemat et al. 2015). However, due to the lack of standardized extraction methods and quantitative evaluation of phytochemicals, it is difficult to compare the results among the available literature (Zhong et al. 2018).

The molecules contained in ABs offer a broad spectrum of actions. This plethora of valuable biocompounds are mainly secondary metabolites, not involved in plant metabolism or vital functions but in other important activities such as defensive action against predators or attraction of pollinators. Plant secondary metabolites are grouped into phenolics, terpenes and alkaloids. Polyphenols comprise a wide range of molecules containing one or more aromatic rings carrying hydroxyl groups, they're largely found in all plant organs (Tufarelli et al. 2017). Phenolic acids are mostly found in leaves, while terpenes in the aerial parts and tannins in roots and seeds (Chiocchio et al. 2021). Phenolic compounds, such as polyphenols, and flavonoids are being referred to, inter alia, as antioxidants preventing oxidative stress through limited generation of free radicals (Deng et al. 2012).

Among bioactive compounds, tannins (which exist in two different forms, the hydrolysed and condensed ones), are found to be effective in disease control and act as health promoters thus are being used as livestock feed additives in substitution for antimicrobial growth-promoting factor (AGP) (Redondo et al. 2014). The AGP administration during long periods in subtherapeutic doses poses a particularly hazardous situation in antimicrobial-resistant microorganism selection, hence their replacement in favour for plant-derived tannins might additionally alleviate the threat to animal welfare and human health other than to food safety. Redondo et al. (2014) reviewed the use of different plant-derived tannins as an alternative to AGP in livestock, considering their growth-promoting and antimicrobial effects. Dietary inclusion of chestnut tannins have been reported to positively affect growth performance, meat quality and antioxidant properties both in poultry and rabbits (Liu et al. 2009; Gai et al. 2010). Their inclusion in broiler diet up to 0.2% improves growth performances and nutrient digestibility (Schiavone et al. 2008). Moreover, the antimicrobial effects associated with the influence on the microbioma balance in the gastrointestinal tract seem to be the most relevant mode of action of tannins (Redondo et al. 2014). More recently, Redondo et al. (2022) found changes in the relative abundance of *Lactobacillus* and *Bifidobacterium* in caecal microbioma of tannins-treated flocks improving gut health. Many studies found that tannin-rich extract inhibits the growth of pathogens like *Campylobacter jejuni* (Gutierrez-Banuelos et al. 2011; Anderson et al. 2012). Tosi et al. (2013), in an *Eimeria* spp. and *Clostridium perfringens* co-infection models, reported a reduction of necrotic enteritis lesions and control of *Clostridium perfringens* load in broilers fed a diet containing a growing inclusion rates of hydrolysed tannins from chestnut extract. In tannins-treated birds infected with *C. perfringens*, Redondo et al. (2022) recorded a reduced incidence of gross lesions in jejunum and ileum compared to a control group.

Lentil and lentil by-products characteristics

Lentil (*Lens culinaris* L.) belongs to the Fabaceae (legumes) family comprising pulses. Total global pulse production in

2022 reached 95,989,881 tonnes, while the same year lentil production was 6,655,827 tonnes accounting for almost 10% of total pulse production (FAOSTAT 2023). They are commonly spread worldwide in human nutrition as a highly valued food because of their nutritional quality such as low fat and high protein contents, and are consumed rather as whole, decorticated, processed or as split seeds (Damte and Tafes 2023). Lentils contain a plethora of bioactive phytochemicals recording the highest polyphenolic content among 8 pulse varieties (Xu and Chang 2007). Pulses' dietary inclusion has gained the interest of researchers based on the increasing consumers' demand for health-promoting diets seeking alternative protein sources (Rebello et al. 2014). Moreover, this trend arises from the correlation between pulses' phytochemicals content with a reduction in the incidence of noncommunicable chronic diseases such as cardiovascular diseases, diabetes and obesity (Duane 1997; Rebello et al. 2014; Zhang et al. 2018).

Lentil seeds can be divided into three distinctive parts from the outside inward: seed coat (8–16%), embryonic axe (1–3%) and cotyledon (80–90%), respectively (Dueñas et al. 2006). Typically, cotyledon contains the main reserve substances (proteins and carbohydrates), while the external coat contains the majority of phenolic compounds (Galgano et al. 2023). The protein content of whole lentil seeds ranges between 20% and 30%, making them an excellent source of plant-based protein, complex carbohydrates, fibres and other trace elements such as minerals, vitamins and phytochemicals (Urbano et al. 2007). Moreover, lentils are reputed to possess a cholesterol-lowering activity due to the high legume fibre content and their starch content represented by 5% rapidly digestible starch and 30% slow digestible starch (Li et al. 2023) that may help to treat common disorders like diabetes. Lentil's amino acid profile presents adequate amounts of essential amino acids, although it regrettably shows a deficiency of sulphurated ones (methionine and cysteine) and tryptophan (Joshi et al. 2017), which require an adequate balance in diet formulation (Mensa-Wilmot et al. 2003; Rebello et al. 2014). Although contained in small amounts, an aspect to be taken into account is the lentil anti-nutritional factors (ANFs) content, mainly protease inhibitors, lectins, saponins, tannins and phytic acid (Blair 2017). Nevertheless, technological treatments (autoclaving, micronization, enzyme addition, etc.) are found to be effective in decreasing pulses ANFs activity (Castell and Cliplef 1990; Wiryawan and Dingle 1999). The reduction of ANF results in a more efficient utilization by livestock species, including monogastric species (Laudadio and Tufarelli 2011; Nalluri and Karri 2021).

Lentils' post-harvest processing is composed of three steps: (1) size-, shape-, density- and colour-dependant mechanical sorting; (2) dehulling, splitting and sorting of the whole or split seeds; (3) milling of seeds, separation of protein and starchy components (Joshi et al. 2017). As a result, different types of by-products are generated, commonly named: lentil straw, screening and hull (bran or chuni), although in the literature lack in a clear differentiation in this latter processing by-product. These by-products may represent a useful source of functional components and phytochemicals, making it worthy to evaluate their dietary supplementation in livestock.

The by-product resulting from the first processing step ('sorting by-product') is the lentils screening consisting of discarded seeds not suitable for human consumption (e.g. damaged, broken or discoloured). Lentils screening by-product (LSB) represents around 2–4% of the total processed lentils (Çabuk et al. 2014) and typically consists of whole and broken lentils, cereal grains, weed seeds, haulm and dust (Hassan et al. 2020). Both Çabuk et al. (2014) and Tsega et al. (2019) pointed out the high nutritive quality of LSB as a valuable animal feedstuff compared to other lentil by-products. Literature reports that LSB may be included at different levels in animals' diet as an alternative protein source instead of SBM (Hassan et al. 2020). Analysis of LSB reported about 26.6% crude protein, 12.4% crude fibre and higher energy content than SBM. Moreover, the referred phytochemical content is 21.01 mg gallic acid equivalent/g DM of phenols, 26.3 mg/100 g DM of saponins, 610 mg/100 g DM of phytic acid and 840 mg catechin equivalent/100 g DM of tannins (Hassan et al. 2020), thus conferring them a potential antioxidant activity (Zhang et al. 2018).

The result of the dehulling process is lentil hulls (bran or chuni), a mixture of broken lentils, seed coats and embryonic axes, representing up to 28% of total lentil (Tiwari and Singh 2012). Studies suggest that pulses polyphenols are essentially contained in the seed coat (Zhong et al. 2018; Galgano et al. 2023) and are responsible for seed coat colour (Irakli et al. 2021). Indeed, dark-coloured lentils (red, bronze and black) contain higher level of polyphenols such as anthocyanins and condensed tannins than pale-coloured ones, exhibiting a significantly higher in-vitro antioxidant activity (Xu et al. 2007). Furthermore, Oomah et al. (2011), using various extraction methods (aqueous ethanol and acetone or hot water stream), found a much higher concentration of phenolic components in lentil hull extract compared to the relative whole seeds. Lentil hulls have been identified as a rich source of dietary fibres and phytochemicals with anti-inflammatory and antioxidant properties, contributing to reduce blood pressure, cholesterol and blood sugar (Dueñas et al. 2006). Moreover, both polyphenols and dietary fibre contained in hulls are proven to positively affect gut health (Mollard et al. 2014). During digestion, lentil hull polyphenols are continuously released into the digestive juice and absorbed by the intestinal tract into the blood thereby improving the intestinal barrier and exerting anti-inflammatory effects (Peng et al. 2022).

Lentil straw is a by-product of threshing lentil seeds and consists of the dry stems and leaves left in the field. Compared to cereal straws, they have higher protein content (7%) (Hailegiorgis and Lemessa 2019), digestible protein concentration, dry matter digestibility and higher metabolizable energy (Sharasia et al. 2017), and thus it can be effectively included in ruminant diet taking into account the specific animals' requirements (Gatenby 2002).

The optimal dietary inclusion level of by-products depends on their chemical and phytochemical composition. Different cultivar, geographical locations, harvesting time and processing methods may influence the chemical composition of lentils and hence by-products. Ciurescu, Vasilachi, Habeanu et al. (2017) analysed the chemical composition of two different lentils' cultivar (Eston vs Anicia) and found a

significant difference in terms of crude fat, crude fibre and crude protein contents. Genotype and environment are also involved in determining differences in phytochemical contents and antioxidant activities, even among the same cultivar (Irakli et al. 2021). Ciurescu, Vasilachi, Ropota et al. (2017) found that the Anicia lentil's cultivar had a significant higher linolenic acid (C18:2) content when compared to the Eston cultivar. This, together with a higher content of alfa-linolenic acid (C18:3) was accompanied by a higher proportion of total polyunsaturated fatty acids (PUFA) both $n-3$ and $n-6$, confirming that lentils possess a valuable fatty acid profile.

Lentil by-products as animal feed

Pulses represent the main crop and staple food in several countries around the world, especially within developing ones (Sharasia et al. 2017). Considering the expected global population growth (TWB 2019) and the consequent increase in global food demand (Sharasia et al. 2017), the pulse sector will most likely undergo through a rapid development to meet the increased demand.

Moreover, pulses use as alternative protein source is expected to further increase in livestock feed industry in the near future as well (Van Barneveld et al. 2000; Laudadio et al. 2009; Wang et al. 2022). Similarly, their by-products can also be considered as a useful resource in livestock feeding. Lentil husk and chuni can be included in livestock ration with no drawback on animal welfare nor growth performance. On the other hand, by-products like straw and other plant parts bear the potential to be included in ruminant nutrition. These LBs are valuable energy and protein sources not in competition with human food production and contributing to decrease the reliance on soybean and cereals in livestock diets (Çabuk et al. 2014; Sharasia et al. 2017; Söğüt et al. 2017; Nalluri and Karri 2021).

Nowadays, attention is paid to the possibility to produce healthier foods of animal origin by intervening on animal nutrition. Thereby, the use of lentil seed coats as animal feed should not be neglected, considering the possibility to enrich the diet with fatty acids and bioactive compounds mainly phenols and tannins (Tolve et al. 2021; Galgano et al. 2023). The nutritional quality of animal products is strongly related to their fatty acid profile and composition. Livestock dietary supplementation with FA may influence the acidic profile of the final product (meat, eggs, milk). Lentil lipids are predominantly polyunsaturated fatty acid (PUFA, from 55.5% to 58.1% of total FAMES) with low content of saturated fatty acids (SFA, from 19.3% to 21.5%). In terms of specific FAs, the most important are the linolenic acid (LA) and alpha-linolenic acid (ALA), because both are essential for humans and animals (Ciurescu, Vasilachi, Ropota et al. 2017). Thus LBs supplementation to livestock may represent a path in ameliorating products' quality.

In animal feeding, the employment of condensed tannins as dietary supplements is becoming popular due to their wide range of biological effects (Gai et al. 2010; Redondo et al. 2014; Zhang et al. 2018; Hassan et al. 2020). Lentils' seed coat, the major by-product of lentil processing, may be considered a potential sustainable source of antioxidant polyphenols (Hassan et al. 2020; Pathiraja et al. 2023). Upon

determination of the tannins content of various fibrous rabbit feedstuffs, Kara (2016) found lentil bran to possess the highest values of condensed tannins, bound condensed tannins and extractable condensed tannins.

Low-level polyphenols and dietary fibre administration in monogastrics have been proven to concur in maintaining gut health, health status and improve performance (Brus et al. 2013; Starčević et al. 2015; Huang et al. 2018; Zhong et al. 2018; Hassan et al. 2020). Whereas in ruminants it has been reported that low-level of tannins upon ruminal microbiome reduced ruminal biohydrogenation of fatty acid with possible consequent increase of unsaturated fatty acid contents in milk and meat.

To the best of our knowledge, limited research has explored the impact of incorporating lentils by-products into animal diets (Sharasia et al. 2017; Biesek et al. 2020).

Lentil straw is proven to have a potential nutritional value, thus can be included in ruminants' rations (Tsega et al. 2019; Islam and Khan 2021; Damte and Tafes 2023). Moreover, the quality of lentil straw is higher than other crop residues (Haile et al. 2016). It shows high fibre content (30–40%) and a protein content up to 10% (Lardy and Anderson 2009). Lentil straw contains less NDF and its ruminal degradability is higher when compared to cereal straws (Abbeddou et al. 2011; Haile et al. 2016; Islam and Khan 2021; Damte and Tafes 2023). Considering the significant average yield of this by-product of 3.5 tons/ha (Hailegiorgis and Lemessa 2019), and its favourable protein content, its use as feed represent a profitable and sustainable way, especially in developing countries and in the dry areas, where livestock are reared on poor pastures (Abbeddou et al. 2011; Haile et al. 2016). Abbeddou et al. (2011), in a study on fat-tailed sheep, compared lentil and barley straw nutritional composition and digestibility, concluding that lentil straw may be used as alternative forage during periods of feed shortage. Mudgal et al. (2018) found that lentil straw can be used as forage or in total mixed ration in goat diet. More recently, Damte and Tafes (2023) reported that feeding of lentil straw can support maintenance requirements of ruminants if properly supplemented.

Lentil screening can be considered as an advantageous alternative feed ingredient for livestock because of its cheapness and nutritive value. Surplus, off quality pulse grains and screening-derived grains show high protein content (22–30% CP on a DM basis) and energy (45–60 Mcal/cwt NEg) thus might be considered as livestock feed (Anderson and Schoonmaker 2006). Lentils contain 21.8% dietary fibre (DM basis), which is higher compared to screenings and seeds (Islam and Khan 2021). Gendley et al. (2002) referred that lentil chuni may be considered as a valid energy and protein feed when included in well formulated and balanced diets for ruminants. Moreover, Gendley et al. (2009) found that when a mixture of 50% wheat bran and 50% lentil bran was fed to bulls, the fermentation in the rumen improved together with a higher protein availability in pulses by-products compared to cereals. Furthermore, pulses' low starch and high non-starch polysaccharide content may reduce the onset of lactic acidosis associated to cereals feeding, making them a good substrate for ruminal fermentation (Van Barneveld et al. 2000). Anderson and Schoonmaker (2006) compared feed intake (FI), weight

gain and feed efficiency among four groups of feedlot cattle. The control group was fed a typical corn and canola meal diet while the treated groups received a pulse grains-based diet (chick peas, field peas or lentils). Throughout the first 3 weeks, pulse grains-supplemented groups showed an ameliorative effect on daily weight gain and increased daily dry matter intake compared to control group. The authors supposed that these results may be related to the higher palatability and protein digestibility of lentils compared to canola or corn. Similarly, Lardy and Anderson (2009) found lentil seeds palatability resulted similar to pea or chickpea, without any changes in growth rate of calves. Also, in beef cattle, diets including lentils, chickpeas or field peas as replacements for maize and canola meal demonstrated similar dry matter (DM) intake and final body weight (FBW) compared to a control group, suggesting that pulse grains could serve as a viable alternative protein source in cattle diet (Gilbery et al. 2007). Furthermore, lambs fed grass hay supplemented with various mixtures of wheat bran and lentil screenings exhibited enhanced growth performance and improved carcass characteristics (Tsega et al. 2019). They concluded that the supplementation of a grass hay basal diet with 227 g wheat bran and 120 g LSB mixture can be considered as feasible for fattening of lambs.

Investigation into alternative protein sources in monogastric species has led to an increased interest in the use of grain legumes in the swine sector as well, since they are considered as a valuable alternative source of protein and energy in monogastric nutrition. Additionally, legumes seeds implementation in monogastric feeding, whether processed or not, may serve as a SBM substitute, reducing reliance on its use and avoiding GMOs inclusion (Sořita et al. 2022; Parrini et al. 2023). The reviewed literature reports the use of pulses in swine as off-grade market seeds. Pulses have been used in swine feed formulation as a complement of cereals due to their chemical and physical characteristics, such as adequate protein and starch content and low fat (Singh et al. 2007; Woyengo et al. 2014). However, to achieve balanced diets, essential amino acids integration is required. According to Castell and Cliplef (1990), methionine supplementation (1 g/kg dietary level) to a 40% lentil-based diet in gilts led to improved meat quality when compared to a traditional SBM-based diet. Regrettably, their inclusion in swine feeding is limited due to the ANFs content of pulse seeds which may possible hinder growth rates, FI and feed utilization, especially in starter pigs (Landro et al. 2012). In this regard, same authors referred no adverse effects on growth rate of starter pigs fed a diet containing up to 225 g/kg of lentil, while higher inclusion rate of 300 g/kg led to reduced daily gain and feed efficiency although without affecting FI (Landro et al. 2012). This might be related to the higher susceptibility of piglets to the ANFs content in pulses (Batterham et al. 1993), which negatively affected digestive functions (Van Heugten 2000).

However, in grower–finisher pigs (23–100 kg BW), dietary administration of up to 300 g/kg (Bell and Keith 1986) or 400 g/kg (Castell and Cliplef 1990) lentil seeds, did not have effects on growth rate. Moreover, according to Mavromichalis (2013), feeding of up to 30% of law lentil seeds can be included in pig diets. Most likely, starter pigs might result more sensitive

to ANF's negative effects than grower–finisher pigs (Batterham et al. 1993; Landero et al. 2012). Nevertheless, the content and activity of these plant secondary metabolites can be reduced via processing methods, improving the nutritive value and utilization efficiency of these feedstuff (Jezierny et al. 2010).

Lentil by-products in rabbits and poultry

The inclusion of lentil by-products (LBs) in poultry and rabbits has been explored as a possible substitute for SBM, aiming to reduce the dependency of this feed ingredient and achieve economic, productivity and sustainability benefits (Ayaşan et al. 2018; Ciurescu et al. 2018; Hassan et al. 2020). Such a shift would also help foster poultry industry development in some areas of the world that cannot afford the supply of soybean meal (Iji et al. 2017). Over the past years, interest in pulses as protein source in poultry feeding have been increasing (Laudadio et al. 2009; Nalle et al. 2011; Dotas et al. 2014; Smulikowska et al. 2014; Zdunczyk, Jankowski, Mikulski et al. 2014; Zdunczyk, Jankowski, Rutkowski 2014; Koivunen et al. 2016; Ciurescu, Vasilachi, Habeanu et al. 2017). When identifying pulses' utilization rates as alternative protein dietary source in poultry, careful consideration has to be given to the amino acid profile, particularly to the lack of sulphur amino acids (methionine and cysteine), that can be overcome through appropriate amino acids supplementation (Wiryanawan and Dingle 1997; Joshi et al. 2017; Nalluri and Karri 2021). Additionally, attention must be paid on the type and quantity of the alkaloids and tannins whose positive biological effects are reversed when administered at high doses, a factor mitigable through adequate processing techniques (Wiryanawan and Dingle 1999; Nalluri and Karri 2021) or through genetic selection of crops. However, chemical analysis assessed tannin content of LSB of about 1.4% (catechin equivalents) (Stanford et al. 1999), an insufficient amount to negatively affect animal performance (Mavromichalis 2013; Suliman et al. 2019; Hassan et al. 2020). In comparison with other legumes, lentils contain relatively small amounts of ANFs such as trypsin and chymotrypsin inhibitors (Guillamoïn et al. 2008; Hefnawy 2011). Therefore, the lentil cultivars studied did not appear to contain harmful levels of ANFs (Ciurescu et al. 2017a).

Nevertheless, dietary inclusion of lentil and LBs in poultry and rabbits should not be considered only as alternative protein source but also as source of dietary fibre, starch, polyphenols and fatty acids that may positively influence gut health, thus improving animal production (Figure 1).

Among pulses, the incorporation of cowpeas into poultry diet has been shown to decrease feed costs and improve production and growth parameters (Chakam et al. 2008; Defang et al. 2008).

Optimal gastrointestinal functionality is essential for sustainable animal production (Celi et al. 2017). Poultry gut health is crucial both for its absorptive role and because of it concurs in maintaining the endogenous metabolic balance, acting as an innate barrier against pathogens (Liu et al. 2009). Rabbits are highly specialized herbivores, being monogastric hindgut fermenters. Their digestive physiology can efficiently convert the proteins contained in cellulose-rich feeds into food containing high-value animal proteins (Dalle Zotte 2014; Cappelli et al.

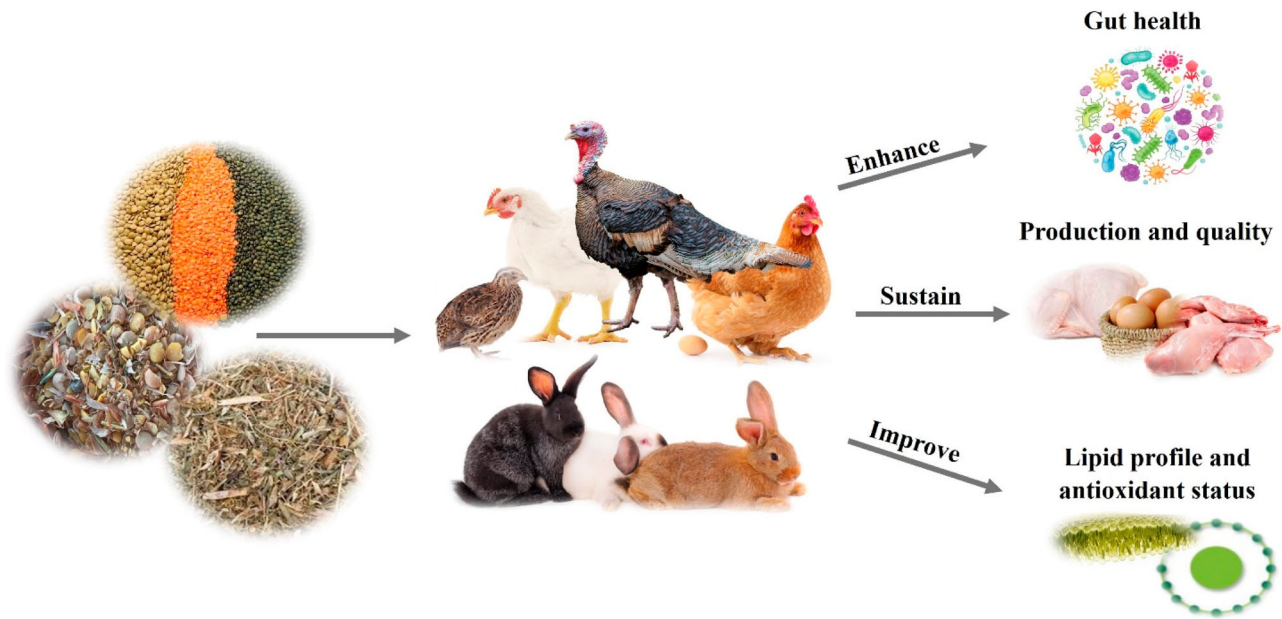


Figure 1. Main effects of dietary lentil and its by-products in poultry and rabbit.

2021; Losacco et al. 2023). Moreover, the fibrous substances of structural carbohydrates act in preserving the gut environment due to their influence on the mucosa physiology and the digesta passage rate, representing a feasible substrate for local microbioma (Kara 2016).

The total dietary fibres (TDF) are the main constituent (up to 50%) of a complete rabbit feed (Alvarez et al. 2007; Gidenne et al. 1998) and comprise both insoluble and soluble fibres. The TDF content of pulses ranges from 14% to 32%, comprising 10–28% of insoluble fibre and 2–9% of soluble fibre (Tosh and Yada 2010; Pathiraja et al. 2023). Lentils bran has a high TDF and water-soluble non-starch polysaccharides content (Kara 2016). These substances are readily fermented in the large intestine (Gidenne et al. 1998) where they sustain the microbioma activity and promote the growth of beneficial bacteria preventing digestive disorders (Alvarez et al. 2007). According to Kara (2016), it was stated that lentil bran, due to its high fibre content and low fermentation ability, may be recommended as a valuable fibrous feedstuff in growing rabbit diet. Lentils showed the second highest starch percentage (47.1%) and a greater percentage of insoluble dietary fibre among pulses (Bednar et al. 2001). Moreover, lentil bran is known as a good source of prebiotics carbohydrates (12.3–14.1% of DM) (Dwivedi et al. 2014) which help in maintaining the gut microbial environment and in preventing gut-associated diseases onset (Ganesan and Xu 2017; Suliman et al. 2019; Hassan et al. 2020). Lastly, lentils are a rich source of phenolic compounds compared to other legumes, which shows its relatively higher antioxidant activity (Kaale et al. 2022). Lentils contain a number of bioactive phytochemicals, such as flavonoids, phytic acid, phenolics, tannins and saponins (Oomah et al. 2011; Paranavitana et al. 2021; Kaale et al. 2022). In physiological conditions, oxidative reactions take place also in the digestive tract (Aruoma et al. 2006) and stress conditions may extend the reactive oxygen species (ROS) production. In a balanced system, oxidative damage is minimized by

endogenous antioxidant defence mechanisms that protect the cell against cellular oxidants and repair systems. Lately, scientific evidence has assessed the balancing effects of dietary antioxidants on the deleterious consequences of the oxidative metabolism when ROS production exceeds the antioxidant defences capacity, such as in stress conditions (McDonald et al. 2001). Notably, dietary inclusion of bioactive compounds like polyphenols enhance the antioxidant defence systems considering their high redox properties to scavenge free radicals and reduce the production of the derived compounds. As a result, the antioxidant activity of polyphenols improves intestinal morphology and functions, better nutrient digestion, absorption and consequent nutrient utilization, hence improving performance indices. In-vitro studies show that lentil hull polyphenols are released in the digestive tract where they may perform anti-inflammatory effects or enhance the intestinal barrier (Peng et al. 2022). Moreover, polyphenols release in the colon can support beneficial gut microbioma metabolism which exert a pivotal influence on various gastrointestinal functions including the defence barrier against enteropathogens (Saura-Calixto 2012).

Recently, Hassan et al. (2020) highlighted LSB as a potential high-quality feed ingredient for growing rabbits due to its nutritive value and phytochemical profile. Indeed, chemical compositional study of LSB showed a significant amount of total phenolic and tannins content suggesting they may act as a functional dietary ingredient in rabbits (Hassan et al. 2020). Moreover, the same study revealed that lentils have high starch and insoluble dietary fibre content and high level of prebiotic carbohydrates that aid to further preserve gut physiology (Dwivedi et al. 2014; Chen et al. 2016; Ganesan and Xu 2017). In addition, Hassan et al. (2020) also investigated the effects of different inclusion levels of LSB (5%, 10% and 15%) instead of SBM on nutrient digestibility, growth performance and carcass characteristics in rabbits. They found a significant higher digestibility of CP (+6%) in the 5% LSB diet

compared to the SBM group, while the 5% and 10% LSB groups showed higher values of digestible crude protein (DCP), total digestible nutrients (TDN) and digestible energy (DE). The authors argued that the improved digestion coefficients of nutrients were related to the protein structures and energy content of LSB. These findings were in accordance with those of Suliman et al. (2019) which reported a significant increase in CP digestibility in rabbits fed a diet with 30% LSB compared to a group that fed a traditional diet with SBM.

The effect of LSB inclusion on rabbits' growth performance and carcass characteristics was also investigated. Hassan et al. (2020) recorded the higher final body weight (FBW) in rabbits fed 5% LSB when compared to a control group, while other inclusion levels (10% and 15% LSB) led to a trend for higher FBW as well, possibly associated with the significantly higher average daily weight gain (DWG). The researchers concluded that LBS could be included in rabbit diet up to 15% without adverse effect on growth performance parameters. Suliman et al. (2019) also reported a positive trend for FBW and DWG with 15% or 30% LSB substitution rates compared to the control group. The same groups also showed a better feed conversion ratio (FCR) reflected by the significant lower daily FI. The results showed no adverse effects on the performance indices of treated rabbits compared to control; moreover, feed costs were lowered by LSB dietary inclusion, which would imply higher economic profitability of these diets. Further, Hassan et al. (2020) concluded that the improvement of growth performance and of feed efficiency indices may be also related with the significant improvement of carcass measurement and meat cuts of rabbits fed LSB diet. These results are in contrast with previous studies (Suliman et al. 2019) where the inclusion of LSB at a level of 15% significantly decreased meat production.

Grain legumes high protein content gain interest in poultry feeding as alternative protein source (Nalluri and Karri 2021). Furthermore, pulses and their by-products could be employed in organic poultry farming to sustain production and development (Nalluri and Karri 2021). In addition, most of the soybean proceeds from genetically modified crops and the trend within European Union is to limit its use in livestock feed due to potential negative effects that the obtained productions could exert on human health (Ciurescu et al. 2017b). The high value of lentils was demonstrated by evaluating the nutritional composition and amino acids digestibility of 15 different feeds, which may be used in organic poultry production as possible substitute of solvent extracted SBM (Grashorn and Ritteser 2016). Although numerous trials investigated grain legumes and their by-products in poultry diet, little is known on the possibility deriving from lentil and LB implementation in this species. As in rabbits, lentils inclusion in poultry diet has been investigated to substitute conventional SBM as the protein source in formulating more efficient, cost-effective and sustainable diets (Adino et al. 2018). Farhoomand (2006) revealed lentil seeds can be included in broiler diet up to 20%, albeit not as the sole protein source to provide balanced diets and avoid amino acid deficiencies. Previously, Yalçin et al. (1991) observed that LBs inclusion had no adverse effects on FI and FCR in poultry. The LBs as SBM substitute at inclusion rates of 5%, 10%, 15% and 20% were investigated to assess the

growth performance, carcass traits and egg yield in quails by Ayaşan et al. (2018). The slightly trend for higher FBW in quails fed 10%, 15% and 20% lentils by-product diets could be related to the improved feed efficiency. On the other hand, Eratak et al. (2022) found no significant differences in FCR and FI among groups of quails fed control or 10%, 15% and 20% LB diets. Similarly, Çabuk et al. (2014) recorded no effects on FI and FCR at 10% and 20% LBs inclusion in laying quails.

In turkeys, LB in the diet up to 15% did not have adverse effect on BW, carcass features and FI, but FCR (Söğüt et al. 2018), demonstrating that it could easily be used in the diet to reduce the cost of feed. The authors also reported that up to 30% of LB may have detrimental effects on growth due to the higher fibrous content of diet (8.8%). Moreover, LBs inclusion rate of 15% (Yalçin et al. 1991) and 20% (Kanat 1992; Kanat and Camci 1993) were found to negatively affect BW and productive performances in quails and laying hens, respectively.

With regard to carcass traits of quails fed LBs, Ayaşan et al. (2018) found no influences on average breast and liver weight at 15% and 20% LBs inclusion rate, although the dressing percentage of LB fed animals was negatively affected by treatments. Likewise, in turkeys, Söğüt et al. (2018) reported that carcass and digestive organs weight were not affected by the dietary inclusion of LB at higher inclusion rate up to 30%. Recently, Eratak et al. (2022) found that LBs inclusion in quail diet had no effect on gizzard, liver, heart or small intestine weight and length; however, 20% LBs inclusion led to significant decrease of pancreas weight, possibly as a consequence of the presence of ANFs such as trypsin inhibitors and tannins.

According to the reviewed literature, the inclusion of LBs in quail diet may influence egg production and quality as well. Their inclusion in laying quail diet at 5% and 10% rate determined increased egg yield, whereas inclusion rates of 15% and 20% negatively affected egg yield percentage (Ayaşan et al. 2018). Similarly, Çabuk et al. (2014) observed a significant increase in egg production with 10% and 20% dietary inclusion of LB. However, the 20% inclusion rate was accompanied by a significant decrease in egg weight. On the other hand, studies revealed that more than 5% LBs to negatively affect hen-day egg production and inclusion rates higher than 10% LBs decreased egg weight (Kiliçalp and Benli 1994), while Kanat (1992) and Yalçin et al. (1991) found 15% LBs in the diet of layers decreased eggs yield, without affecting egg quality. Interestingly, Çabuk et al. (2014) found significant dietary effects derived from LBs inclusion in yolk parameters of treated quails, except for the redness parameter of yolk. The lightness was increased with LBs dietary inclusion up to 10%, while the yellowness was increased by the 20% inclusion rate, but not by 10%. Similarly, Ayaşan et al. (2018) observed linear increase in yolk colour of LBs-fed laying quails at increasing inclusion rates. These findings demonstrated that LBs dietary inclusion may increase the deposition of yellow pigment in the yolk. In consideration of consumer preference on yolk colour, LBs could be used to improve the yellowness (Ayaşan et al. 2018). Lentil seed coat, the major processing by-product, may be considered a sustainable source of polyphenols with high natural antioxidant activity (Dueñas et al.

2006; Oomah et al. 2011; Peng et al. 2022; Pathiraja et al. 2023). The current research status regards this by-product is most focused on the phenolic composition and in-vitro antioxidant activity evaluation, on the other hand, to date, studies in vivo are very scant (Peng et al. 2022). Çabuk et al. (2014) investigated the antioxidant effect of LBs dietary inclusion in laying quails, analysing the level of lipid oxidation by yolk thiobarbituric acid (TBA) content measurement at 0, 7, 14, 21, 28 days of storage. They found that the levels of lipid oxidation to linearly decrease according to the increasing dietary inclusion of LBs, but not depending on the storage time. Literature links up the lentil's antioxidant activity with its tannins' contents; at this regard, Çabuk et al. (2014) suggested that these compounds could be responsible for the increased egg oxidative stability.

In broilers, Ciurescu et al. (2017a) evaluated the effects on growth performance, carcass characteristic and caecal pH after dietary inclusion of two lentil seeds' cultivars (Anicia vs Eston) at 20% and 40% levels compared to a control diet with SBM. The broilers fed lentil seeds had comparable productive performance and caecal pH among the groups. The group fed the experimental diet with the Anicia cultivar showed a significant increased weight of the small intestine. Authors concluded that this effect could be related to the high level of dietary fibre (Van der Klis and Von Vorst 1993; Iji et al. 2001) and the improvement of gut epithelium features and functions (Ciurescu et al. 2017a). In addition, both lentil seeds cultivar showed a trend to an increase of carcass and abdominal fat weight of broilers. In conclusion, the replacement of SBM with lentil seeds at inclusion level up to 20% showed similar effects.

Raw lentil seeds' dietary inclusion has been found to influence the meat fatty acids profile in broilers, thus improving meat quality (Ciurescu et al. 2017a). Fatty acids (FAs), especially essential FAs, are gaining importance in poultry feeding systems not only for improving the welfare and productivity of birds but also because of the increasing trend among consumers towards balanced diets to minimize adverse health issue. Evidence has shown that contents of PUFAs and favourable fatty acids profile in poultry products may be enhanced by dietary manipulation (Alagawany et al. 2019). Further, performance indices of broilers are improved through the supplementation of FA or their sources. In terms of lipids content, Ciurescu, Vasilachi, Habeanu et al. (2017) found that lentils seed to contain mainly polyunsaturated fatty acid (PUFA, from 55.5% to 58.1% of total FAMES, depending on cultivar) with a low content of saturated fatty acids (SFA, from 19.3% to 21.5%). Moreover, Zhang et al. (2014) attested that linoleic acid (LA) (C18:2) was the dominant FAs ranging from 40.73% to 47.06%, followed by oleic acid (C18:1; 20.11–28.0%), palmitic acid (C16:0; 12.67–14.82%) and alpha-linolenic acid (ALA) (9.00–13.28%). Ciurescu, Vasilachi, Habeanu et al. (2017) assessed the dietary effect of lentils on the composition of FAs in the breast muscle of broilers that fed two different lentil cultivars at two inclusion levels (20% and 40% of diet) compared to traditional SBM-fed group. Results showed that the meat of LBs-supplemented broilers detected a significant higher level of $n-3$ PUFAs and a consequent reduction in the ratio $n-6/n-3$. In particular, experimental diets resulted in a significant increment in eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and

docosahexaenoic acid (DHA) in comparison with the control group. According to the same authors, this legume could substitute SBM in the consideration of the significant increased level of ALA found in broilers fed lentils.

Conclusion and perspectives

Existing scientific literature highlights that the inclusion of lentil and lentil by-products in poultry and rabbit diets as a valuable, cost-effective and environmentally sustainable feed source is of growing interest but needs deeper investigation. Indeed, integrating agricultural by-products into livestock rations present an opportunity to valorise these materials while mitigating disposal concerns. The reviewed literature examined various aspects and considered different lentils by-products and inclusion levels. Future studies should focus on determining the optimal inclusion level to maximize beneficial effects in livestock species, including poultry and rabbits. Moreover, lentil by-products dietary inclusion could result in more efficient feed utilization from livestock in countries where these are produced at high yield ultimately preventing dependence on imported feeds.

Lentils are considered a promising legume to be included in livestock diet due to the high content of functional molecules with health-promoting activity. As discussed, incorporating lentil by-products into diet can reduce reliance on soybean meal, thereby lowering feeding costs. Additionally, their consumption is associated with improvements of animal growth and productivity. However, further research is needed to evaluate the effects of lentil by-products' nutritive value and phytochemical composition on animal health, oxidative stress and inflammatory status.

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