Peter Haščík, Matej Čech, Juraj Čuboň, Marek Bobko,
Miroslava Kačániová, Henrieta Arpášová

Effect of supplemental flax and pumpkin pomace on meat performance and quality of Ross 308 broiler chickens meat

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Name: Effect of supplemental flax and pumpkin pomace on meat performance and quality of Ross 308 broiler chickens meat

Authors: **prof. Ing. Peter Haščík, PhD.**

Institute of Food Science,

Faculty of Biotechnology and Food Sciences,

Slovak University of Agriculture in Nitra

Ing. Matej Čech

Institute of Food Science,

Faculty of Biotechnology and Food Sciences,

Slovak University of Agriculture in Nitra

prof. Ing. Miroslava Kačániová, PhD.

Institute of Horticulture,

Faculty of Horticulture and Landscape Engineering,

Slovak University of Agriculture in Nitra

prof. Ing. Juraj Čuboň, CSc.

Institute of Food Science,

Faculty of Biotechnology and Food Sciences,

Slovak University of Agriculture in Nitra

assoc. prof. Ing. Marek Bobko, PhD.

Institute of Food Science,

Faculty of Biotechnology and Food Sciences,

Slovak University of Agriculture in Nitra

assoc. prof. Ing. Henrieta Arpášová, PhD.

Institute of Animal Husbandry,

Faculty of Agrobiology and Food Resources

Slovak University of agriculture in Nitra

Reviewers: **prof. Ing. Stanislav Kráčmar, DrSc.**
College of Business and Hotel Management, Brno
Czech republic

prof. Ing. Marcela Capcarová, DrSc.
Institute of Applied Biology,
Faculty of Biotechnology and Food Sciences,
Slovak University of Agriculture in Nitra

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P≥0.05 nepreukazný rozdiel na hladine významnosti 0,05

$\overline{x}$ priemer

% percento

P≤0.05 preukazný rozdiel na hladine významnosti 0,05

± plus, mínus

a i. a iné

ad libitum do sýtosti

Ca vápnik

Cl chlór

cm centimeter

Cu meď

ES Európske spoločenstvo

et al. a kolektív

EÚ Európska únia

Fe železo

g gram

IU medzinárodná jednotka

J jód

JOT jatočne opracované telo

C kontrolná skupina

K draslík

kg kilogram

kJ kilojoul

KKZ kompletná kŕmna zmes

KTJ kolónie tvoriace jednotky

l liter

ME_N metabolizovateľná energia opravená na dusíkovú rovnováhu

Mg horčík

mg miligram

MJ megajoul

ml mililiter

Mn mangán

Na sodík

NL dusíkaté látky

P fosfor

P preukaznosť rozdielov

resp. respektíve

SD smerodajná odchýlka od priemeru

Se selén

t tona

t.j. to jest

Zn zinok

INTRODUCTION

Flax is one of the oldest cultivated crops. Its fruit is an oilseed used mainly in the food industry to produce various health-promoting products. Flaxseed contains many biologically active compounds and elements, including linolenic acid, linoleic acid, lignans, cyclic peptides, polysaccharides, but also fewer desirable alkaloids, cyanogenic glycosides and cadmium. However, the greatest attention is paid to extracts containing α-linolenic acid or lignans. Overall, the consumption of whole flax seeds is recommended for the content of mucus, protein and especially oils with a favorable fatty acid composition. Flaxseed oil is known as a rich source of omega-3 fatty acids and is therefore often used as a vegetable substitute for fish oil. Flax pomace is a by-product in the production of linseed oil with a relatively low energy value but with a non-negligible protein content. Due to the presence of antinutritional compounds such as phytic acid, linatin and cyanogenic glycosides, flax pomaces have limited use in foods despite their high protein content. Cyanogenic compounds, especially cyanogenic glycosides, can be degraded to toxic HCN after ingestion. Therefore, pomace high in fiber and protein with high nutritional potential have been underused and of limited use, at least as a feed supplement for livestock.

Unlike flax seeds, pumpkin seeds are usually considered an industrial waste product and discarded. Pumpkin seeds are often used as uncooked, cooked, dried, or roasted for home consumption. After pressing, oil pomaces are poor in fat but rich in protein, fiber, minerals such as iron, zinc, calcium, magnesium, manganese, copper and sodium, polyunsaturated fatty acids, phytosterol and vitamins. Except for the production of seeds for direct consumption, pumpkin seeds, and in particular their pomaces, are considered a cheap by-product and their incorporation into food can increase nutritional value at low cost. Their positive effects on overall health, blood glucose, cholesterol, immunity, liver function, gallbladder, prostate health problems, depression, inflammation, cancer treatment and parasite inhibition are observed. Incorporating these agro-industrial waste products back into the food chain is one of the steps towards food sustainability. One of the possibilities for their use is also application to feed rations of livestock, as animal production is constantly growing. The application of pumpkin seeds or pumpkin pomace could have a positive effect on the nutritional composition of animal products and thus on the health of consumers.

At the beginning of the new millennium, livestock production in the European Union faced many challenges, such as a ban on the use of meat-and-bone meal due to the BSE epidemic in the UK in the 1990s, but especially a ban on the use of feed antibiotics and growth hormones in feed rations, which continue to be used worldwide. As it was still necessary to pay attention to the quality of animal products and the overall safety of the food chain, it was important to start looking for various natural alternatives to previously used antibiotics such as probiotics or plant extracts, etc., which would stimulate growth. They would not have adverse side effects on animal health, would not burden their organs and would not leave residues in animal products intended for human consumption. At the same time, alternative natural feed additives would reduce the risk of bacteria developing antibiotic resistance, which was the main reason why their use was banned.

The administration of natural feed additives in poultry is a suitable prevention in individual stages of life to support viability and growth, before and during increased physical activity, in stressful situations, under thermal stress, to support laying, increase growth and reduce mortality. Their use is also recommended to support the treatment of infectious diseases and intoxications, or after any necessary antibiotic treatment.

Poultry meat is an important component in human nutrition. Broiler chickens have a dominant share in the consumption of poultry meat (90%). In connection with the consumption of chicken meat, the issue of a healthy diet includes several aspects, such as low cholesterol, appropriate representation of fatty acids and others. According to statistics, poultry meat is generally popular, and its production and consumption is constantly growing, which is also since there are no religious and social restrictions on its consumption. In addition, thanks to the undemanding and short rearing, it is relatively cheap and easy to cook. However, with its growing consumption, there is also pressure on feed production, which is a prerequisite for a profitable breeding economy and the basis for quality animal products. For mentioned reasons, ways have been sought in recent years to incorporate large by-products of the food industry into livestock nutrition and thus return them to the food chain. Undoubtedly, these are also pomaces and flours produced during the processing of oilseeds, which are rich in fiber, protein, and polyunsaturated fatty acids. This scientific monograph is devoted to the above-mentioned flax and pumpkin pomaces.

1 LITERATURE REWIEV

1.1 Flax (*Linum usitatissimum*)

1.1.1 Flax characteristics

Flax is one of the oldest crops cultivated both for oil and fiber production and with wheat and barley laso one of the first used by mankind for basic life requirements and agriculture processing **(Hajnalová, 1999).** The archaeological evidence points that flax was cultivated back to >6000 BC and it is considered as one of the oldest and most useful crops **(Jhala and Hall, 2010)**. Although it was used mainly as source for textile industry in the past, nowadays it is generally known as healthy, enviromental-friendly, and natural product in food industry **(Nôžková et al., 2016)**.

Flax as a whole plant is one of the oldest crops grown for fiber production and its seeds for oil production. It belongs to the genus *Linum* and the family *Linaceae*. The botanical name *Linum usitatissimum* gave flax to Linnaeus in his book "*Species Plantarum*" **(Linnaeus, 1857)**. This annual herb has a shallow root system. The common names flax and flaxseed are used for *L. usitatissimum* in North America and Asia. Depending on the purpose, varieties intended for either fiber or oil production were gradually bred **(Millam et al., 2005)**. While those grown for seeds or for oil production are lower, have more branching and thus more seed capsules, varieties for flax fiber production are less branched, are significantly taller and coarser **(Jhala and Hall, 2010)**.

It is estimated that flax comes from the Mediterranean and South-West Asia **(Millam et al., 2005)**; but the exact location is unknown. The original reason for growing flax is also uncertain but based on archaeological evidence it is estimated that flax was primarily grown for the production of fabrics. A comparative study of the genetic diversity of the stearoyl-ACP desaturase II locus from *L. usitatissimum*, *L. angustifolium* indicates reduced diversity in cultivated species, suggesting that flax may have been originally grown for oil production **(Allaby et al., 2005)**. Although flax is grown in smaller quantities worldwide compared to other oilseeds, but for its adaptability it is grown in different climates and used in different products. Due to its unpretentiousness, it can also be used in the production of various organic foods. Researchers all over the world are currently researching such products **(Jhala and Hall, 2010)**.

Two types of flax are commonly grown in Europe - for seed production and for textile production. These differ mainly in botany, morphogenesis and paleontogenesis, climate requirements, cultivation methods, methods, and time of harvest, if necessary special requirements if grown in organic farming **(Heller et al., 2015)**. An interesting type is flax with a dual purpose, which has favorable yields of fibers and seeds **(Smyikal et al., 2011; Melnikova et al., 2014)** and is considered more economically stable to market changes compared to varieties grown exclusively for fiber production **(Zając et al., 2012)**. It is also important to pay attention to the selection of the right varieties for specific growing and climatic conditions, which significantly affect the overall harvest **(Soto-Cerda et al., 2014)**. Crop emergence also depends on the selected genotype (cultivar) **(Kurt and Bozkurt, 2006)**.

After harvesting, flax seeds intended for the food industry are sieved through fine-mesh sieves to ensure a balanced and clean batch of seeds (ideal purity is 99.9%). Flax seed has a flat, oval shape with a pointed tip. Compared to sesame seeds, it is slightly larger, measuring about 4–6 mm **(Daun et al., 2003)**. The seeds are brittle, chewable with a pleasant nutty taste **(Carter, 1996)**. The color of flax seeds varies according to the variety from dark to light yellow **(Daun et al., 2003)**; the color is determined by the amount of pigment in the outer shell (the more pigment, the darker the seed). The color variations of flax seeds can be modified in various ways, but mostly by breeding techniques. The most common type of flax, which is also rich in omega-3 fatty acid – alpha-linolenic acid (ALA) is brown flax, while yellow flax is known mainly in two varieties – Omega, which is as rich in ALA as brown flax, and the variety Solin, which has a low content of ALA and was primarily bred to produce margarines due to high yields **(Morris, 2007)**.

Brown flax and yellow Omega are commonly sold in health food stores, some supermarkets and online. The Solin variety is generally intended for industrial processing and is not sold directly to consumers. In addition to the production of margarines, this variety is used, for example, as an ingredient in certain wholemeal breads sold in Australia and the United Kingdom. This variety must always be clearly marked so that it can be more easily recognized by growers and processors. A promising new type of flax is NuLinTM containing more ALA than traditional flax. All flax varieties grown for human consumption or other purposes were developed using traditional plant breeding methods and do not contain GMOs **(Morris, 2007)**.

The terms “flaxseed” and “linseed” are often used as the same, although in Europe, the term “flaxseed” describes the varieties grown for making linen, while the North Americans use "flaxseed" to describe flax when it is eaten by humans and "linseed" to describe flax when it is used for industrial purposes, such as linoleum flooring **(Morris, 2007)**.

1.1.2 Nutritional composition of flax seeds

Flaxseed is a multicomponent system with bio-active plant substances such as fats with desirable fatty acids composition, protein, dietary fiber, soluble polysaccharides, lignans, phenolic compounds, vitamins (A, C, F, and E), and minerals (P, Mg, K, Na, Fe, Cu, Mn, and Zn) **(Jheimbach and Port Royal, 2009)**. The basic composition of flaxseed is shown in Table 1; and the components of flaxseed are discussed below.

Table 1: Chemical composition of nutrients and phytochemicals in flaxseed **(Goyal et al., 2018)**

Nutrients/Bioactive Compounds	**Quantity/100 g of Seed**	**Nutrients/Bioactive Compounds**	**Quantity/100 g of Seed**
Carbohydrates	29.0 g	Biotin	6 mg
Protein	20.0 g	α-Tocopherol	7 mg
Total fats	41.0 g	δ-Tocopherol	10 mg
Linolenic acid	23.0 g	γ-Tocopherol	552 mg
Dietary fiber	28.0 g	Calcium	236 mg
Lignans	10–2600 mg	Copper	1 mg
Ascorbic acid	0.50 mg	Magnesium	431 mg
Thiamin	0.53 mg	Manganese	3 mg
Riboflavin	0.23 mg	Phosphorus	622 mg
Niacin	3.21 mg	Potassium	831 mg
Pyridoxin	0.61 mg	Sodium	27 mg
Pantothenic acid	0.57 mg	Zinc	4 mg
Folic acid	112 mg		

Lipids, proteins, and carbohydrates

Flaxseed contains approximately 45% lipids and 55% other substances in the dry matter. Most fats are stored in cotyledons (75%) and the remaining 22% and 3% are distributed in the semen envelope and embryo, respectively. Fats are the main source of energy for the germination of flax seeds. From a microscopic point of view, flaxseed lipids are deposited in droplets with a diameter of 1.3 μm, which contain 98% of neutral lipid, 1.3% of protein or oleosin proteins in the coating layer, 0.9% of phospholipids and 0.1% of free fatty acids **(Daun et al., 2003)**.

From a nutritional point of view, flax fats are its most attractive component of flaxseed due to the favorable ratio of fatty acids, but especially the high content of polyunsaturated fatty acids, namely ALA (about 55%) and linoleic acid (about 14%), and an adequate proportion of MUFA, especially oleic acid (18%) **(Carter, 1993)**. Flaxseeds also contain small amounts of saturated fatty acids, such as palmitic acid (5%) and stearic acid (3%). In addition to fatty acids, the lipid composition of flax also includes small amounts of vitamin E group derivatives (e.g., tocopherol and tocotrienol), sterols, and carotenoid pigments such as lutein **(Daun et al., 2003)**.

Most proteins are also stored in flaxseed cotyledons and serve as the main source of nitrogen during germination **(Wanasundara and Shahidi, 2003)**. Flaxseed contains 20–30% crude protein, which does not correspond to the actual amount of protein, as it also includes non-protein nitrogen (NPN) in the form of various vitamins, sinapine, choline and cyanogenic glycosides. After subtracting non-protein nitrogenous substances, the pure flax protein content is approximately 18% **(Daun et al., 2003)**. Flax protein has a similar amino acid composition as soy; it contains high levels of asparagine, glutamic acid, leucine, and arginine **(Oomah et al., 1993)**, but is poor in sulfur-containing essential amino acids, methionine, and cysteine **(Hall and Shultz, 2001)**. The percentage of essential amino acids is approximately 36% of the total amino acid content. Although it is significantly less compared to animal proteins, the percentage of flax fatty acids still gives great potential for its use as a source of protein **(Wanasundara and Shahidi, 2003)**.

Flaxseed proteins can be separated into a high molecular weight fraction and a low molecular weight fraction using size exclusion chromatography. 64–66% of the flaxseed proteins are high molecular weight fractions, often referred to as linin. On the other hand, the major component of the low molecular weight component of flax protein is called collinin, which makes up about 42% of total flaxseed proteins **(Lei et al., 2003)**. Flaxseed pomace dissolved in distilled water, was mixed with ultrazyme, alcalase and viscozyme, at 50 °C, 1.5 h, agitation at speed of 200 rpm and resulted in 152 g of protein yield from 1 kg of seed pomace **(Ribeiro, Barreto and Coelho, 2013)**. Flax protein isolate was extracted by isoelectric-precipitation at pH 8.5 using 1.0 M NaOH, followed by precipitation at pH 3.8 **(Karaca, Low**

and Nickerson, 2011). Salt-precipitated protein isolates had higher protein solubility compared to flax proteins extracted from isoelectric precipitated isolates. Combining an alkaline protein separation step followed by cellulase and ethanol precipitation resulted in protein isolate with high protein content (86.8%) compared with isoelectric protein precipitation or cellulase treatment alone (51.1 and 65.1%, respectively) **(Tirgar et al., 2017)**.

Comparing flax protein isolates to canola protein isolates, it had higher emulsifying activity index **(Karaca et al., 2011)**. **Kaushik et al. (2016)** produced 90% flax protein isolate (FPI) through demucilaging at 60 °C. The protein isolate had a higher emulsion stability index (375.5 $m^2.g^{-1}$) compared to other proteins such as soy, whey, and gelatine. Moreover, at low pH, the FPI stabilised emulsions were more stable compared to the other tested proteins. The low lysine to arginine ratio (0.25) of FPI also make it a suitable ingredient for heart-healthy foods **(Kaushik et al., 2016)**. FPI also has a high level of arginine and sulphur amino acids (cysteine) what makes it a useful protein ingredient for low weight at birth infant formulas **(Wang et al., 2006)**.

Flaxseed carbohydrate is concentrated in the hull **(Oomah et al., 1996)**. Flaxseed carbohydrate consists of indigestible carbohydrate, often referred to as dietary fibre, and a small proportion of digestible carbohydrate. Flaxseed contains less than 1–2% of digestible carbohydrate, mostly presents in the form of soluble sugar **(Bhatty and Cherdkiatgumchai, 1990)**. The bulk of flaxseed carbohydrate is indigestible and consists of soluble fibre and insoluble fibre. Soluble fibre of flaxseed is commonly called flaxseed mucilage, or less commonly as viscous fibre and flaxseed gum. Flaxseed mucilage is in the outermost layer of flaxseed hull, and it is easily leached out to form a viscous layer when the flaxseed is wetted **(Susheelamma, 1987)**. This mucilage accounts for about a quarter of the total carbohydrate in flaxseed, accounting for approximately 7–10% of the total seed composition **(Carter, 1993)**. However, recent studies on New Zealand flaxseed found mucilage contributed 60–64% of the carbohydrates fraction depending on the fractionation method used for separation of mucilage **(Tirgar et al., 2017)**. The insoluble part of flaxseed carbohydrate consists of non-starch polysaccharides, mainly cellulose and lignan **(Arion Vaisey-Genser and Morris, 2003)**.

Structural studies using viscometry and light scattering methods revealed that flaxseed mucilage is composed of heterogeneous polysaccharides with stiff random coil structure **(Goh et al., 2006)**. Flaxseed mucilage is a mixture of polysaccharides composed of L-galactose, D-xylose, L-arabinose, L-rhamnose, and D-galacturonic acid, and a trace of D-glucose based on an acid hydrolysis analysis **(Bemiller, 1986)**. Flaxseed mucilage can be further separated into two groups: a neutral fraction (83%) and an acidic fraction (17%) **(Dev and Quensel, 1988; Warrand et al., 2005)**. The neutral fraction consists of arabinoxylans (56%) and galactoglucans (44%). This neutral fraction of flaxseed mucilage is composed of D-xylose (62.8%), L-arabinose (16.2%), D-glucose (13.6%), and D-galactose (7.4%). The acidic fraction of the mucilage contains two heterogenous pectin-like molecules composed of L-rhamnose (54.5%), D-galactose (23.4%), and L-fucose (10.1%), together with a small portion of D-xylose (5.5%), L-arabinose (2.0%), and D-glucose (4.5%) **(Warrand et al., 2005)**.

Flaxseed pomace is rich in mucilage or dietary fibre, which can be extracted by alkali aqueous solution at pH 12, followed by centrifugation to collect the supernatant **(Gutiérrez et al., 2010)**. The sticky mucilage solution can be freeze-dried, spray-dried or vacuum dried to generate a shelf-stable powder product. The mucilage can be used as a thickening and emulsifying agent in food, pharmaceutical and cosmetic applications due to its desirable physical properties. Apart from its physical properties, several health benefits have been attributed to flax mucilage. For example, it can be used as a dietary fibre source in human nutrition or serves as a prebiotic for the healthy growth of gut flora. Mucilage also can be used in fermented dairy products to support the growth of lactic acid bacteria and favourably modify the product texture at the same time **(HadiNezhad et al., 2013)**. In addition, in vivo studies showed that flax mucilage has anti-ulcer activity and it significantly reduced the number and length of gastric ulcers induced by ethanol in rats **(Dugani et al., 2008)**. Polysaccharides or oligosaccharides from flax seed cake were reported to possess anti-tumour as a result of their antioxidant properties **(Gutiérrez et al., 2010)**.

The authors suggested that the anti-radical activity of the saccharides prevents the oxidation of proteins, lipid or DNA, thus preventing the leading causes of cancer. Chitooligosaccharides from flax seed cake demonstrated antimicrobial properties against pathogenic bacteria and fungi such as Candia albicans, Penicillium chrysogenum, Fusarium graminearum and Aspergillus flavus **(Xu, Hall and Wolf-Hall., 2008)**.

Phenolic compounds

A wide range of plants contains phenolic compounds. These compounds are often associated with various health benefits, mainly due to their antioxidant activity. Polyphenols such as phenolic acids and flavonoids exhibit therapeutic properties such as anti-microbial, anti-inflammatory, anti-thrombotic, anti-allergenic, anti-atherogenic, antioxidant, anti-cardiovascular disease, and vasodilatory effects **(Balasundram, Sundram and Samman, 2006)**. Phenolic compounds in flaxseed can be classified into simple phenolic acids and the more complex lignans. Canadian flaxseed was reported to contain 790–1030 mg.100 g^{-1} phenolic acids **(Oomah et al., 1996)**. This value is dependent on cultivar and environment. Phenolic acids in flaxseed are mainly composed of phydroxybenzoic acid with a significant amount of chlorogenic acid, ferulic acid, and coumaric acid **(Taylor, 2011)**. The increased phenolic acids contents in flaxseed extract after oil removal indicates that these compounds are in the non-lipid phase of flaxseed. Extraction of polyphenols can be achieved by 50% aqueous ethanol, a solid-liquid ratio (1:60), in a shaker for 30 min at 200 rpm at 25 °C **(Gutiérrez et al., 2010)**.

Lignans are diphenolic compounds containing a 2,3- dibenzylbutane skeleton, which are concentrated in the seed coat fraction of flaxseed **(Madhusudhan et al., 2000)**. Secoisolariciresinol diglucoside (SDG) is the main flaxseed lignan with 610–1300 mg of SDG presents in 100 g of flaxseed **(Johnsson et al., 2000)**. Other lignans, such as matairesinol and pinoresinol, are also present in minor levels in flaxseed **(Thompson et al., 1991; Meagher et al., 1999)**. The abundance of lignans in flaxseed compared to other plants has made lignans as the focus in many studies on the phenolic compounds in flaxseed **(Thompson et al., 1991)**. **Renouard et al. (2012)** reported the extraction of lignans can be achieved by hydrolysis with hydrochloric acid followed by an extraction with a mixture of ethyl acetate and hexane. Another method reported by these authors to extract from flax hulls was to use 70% aqueous methanol for under continuous stirring.

The acknowledgement of the health benefits of flaxseed lignan has increased recently. Plant lignans per se do not provide health benefits for human, however they provide the precursors for the development of mammalian lignans which have protective effects against chronic diseases including cancer, cardiovascular disease, diabetes, and kidney disease **(Prasad et al., 2003)**. Plant lignans are converted to the beneficial mammalian lignans enterodiol and enterolactone by the natural microflora in human colon. The formed mammalian lignans are then absorbed in the colon, transported to the liver, and secreted in the bile **(Setchell, 1995)**. Some of these compounds will reach the kidney and are then excreted along with urine. The production of beneficial ammalian lignans was directly related to the amount of consumed flaxseed **(Nesbitt and Thompson, 1997)**.

Flax cake lignans have shown different preventive effects on various cardiovascular complications such as atherosclerosis, hyperlipidemia, ischemia, hypertension, and cardiotoxicity, which has also been supported by preclinical and clinical studies (**Zanwar, Hegde and Bodhankar 2011; Zanwar et al., 2014)**. In principle, polar solvents, such as aqueous methanol or ethanolic solvents, were used as the first step in the extraction of lignan from flax pomace. The yield of lignans was increased after the incorporation of a dilute hydrochloric or acetic acid treatment step after initial extraction with aqueous methanol or ethanol, followed by extraction with ethyl acetate: hexane **(Meagher et al., 1999; Charlet et al., 2002)**. Recently, direct aqueous acid extraction followed by extraction with ethyl acetate: hexane has been found to be optimal for the extraction of flax lignans **(Lehraiki et al., 2010)**.

Antinutrients

Just like some other plants, flaxseed contains anti-nutrients which may be harmful to human health. The main antinutrients found in flaxseed are phytic acid, linatine, and cyanogenic glycosides. Fortunately, no adverse effect, including food poisoning, due to flaxseed consumption has been reported in the literature **(Daun et al., 2003)**.

Phytic acid

Phytic acid is a phosphorus-rich compound with a very strong chelating capability to mono- or divalent mineral cations such as potassium, magnesium, iron and zinc (Thompson et al., 1989). Phytic acid contains 60–90% of seed phosphorus, making this compound important in germination and seedling growth **(Daun et al., 2003)**. The amount of phytic acid in flaxseed varies from 0.80% to 1.50% of the dry seed weight, which is comparable to phytic acid concentrations in peanut and soybean **(Oomah et al., 1996)**. The role of phytic acid in animal and human nutrition has not been completely elucidated **(Daun et al., 2003)**. Because of its strong chelating properties, phytic acid may cause zinc, calcium, and iron deficiency **(Harland and Morris, 1995)**. However, this negative effect is likely to be dose dependent as rats fed with a small amount of flaxseed did not show zinc deficiency **(Ratnayake et al., 1992)**. In contrast, phytic acid may have a positive effect in reducing blood glucose level and colon cancer incidence **(Daun et al., 2003)**. The issue of phytic acid may become more important if high amounts of flaxseed were consumed regularly.

Linatine

Linatine is a polar compound with an amine moiety located mostly in the cotyledons of flaxseed. Flaxseed contains 100 ppm of linatine **(Klosterman et al., 1967)** although this value might have been underestimated **(Daun et al., 2003)**. The role of linatine as an anti-nutrient compound was discovered after the occurrence of vitamin B6 deficiency symptoms, such as loss of appetite, poor growth, nervous disorders, and anaemia, in flaxseed fed chicks **(Kratzer, 1946)**. In human, linatine did not affect vitamin B_6 level and metabolism in people fed up to 50 g of ground flaxseed per day **(Ratnayake et al., 1992)**. The recommended nutritional guideline regarding linatine is to include enough vitamin B_6 in the diet from other food to suppress the anti-nutritional effect of linatine in flaxseed **(Daun et al., 2003)**.

Cyanogenic glicosides

Cyanogenic glycosides are commonly found in many plant species such as almonds, wheat, barley, sorghum, cassava, apples, stone fruits, but also flax seeds **(Cho et al., 2013)**. In plants, they occur in the glycosyl form – cyanogenic glycosides (CG). Only small amounts are present in the form of non-glycosidic cyanogens (NGC) **(Wanasundara and Shahidi, 1998)**. The presence of four types of cyanogenic glycosides has been described in flaxseed: linustatin, neolinustatin, linamarin and lotaustralin. Linamarin and lotaustralin are monoglycosides, while linustatin and neolinustatin are diglycosides. Their toxicity lies in the fact that they can decompose into highly toxic hydrogen cyanide (HCN). Its amount in CG-containing plants is negligible under normal physiological conditions **(Wanasundara and Shahidi, 1998)**. However, hydrogen cyanide can be released from nuclides by acidic or enzymatic hydrolysis, for example by hydrolysis by intestinal β-glycosidases/acidic environment in the digestive tract, releasing toxic hydrogen cyanide **(Bacala and Barthet, 2007)**. The toxicity of hydrogen cyanide lies in respiratory distress, endangerment of the endocrine, cardiovascular and nervous systems **(Cheeke, 1990)**. During CG degradation, thiocyanates responsible for iodine deficiency disorders such as goiter and cretinism may also be formed **(Delange, 1994)**. It is estimated that 100 g of flaxseed can release 19 – 100 mg of hydrogen cyanide equivalent **(Daun et al., 2003)**.

Haque and Bradbury (2002) state that 100 g of crushed flax in the form of scrap from the New Zealand variety contained 21 mg of hydrogen cyanide equivalent, which corresponds to its daily release of 5–10 mg HCN (at the recommended dose of flax 1–2 tablespoons). However, this is still below the acute toxic dose for adults of 50–60 mg HCN and less than the lethal dose for humans of 30–100 mg.day^{-1} **(Roseling, 1994)**. Therefore, the positive aspects of flax consumption are in an advance **(Haque and Bradbury, 2002)**. While the mentioned recommended dose of flax 1–2 tablespoons per day contains only 5–10 mg of hydrogen cyanide, flax pomace in the same amount after obtaining flaxseed oil contain up to twice the amount of HCN. It follows from the above that only 6 tablespoons of flax pomace are sufficient to achieve an acute toxic dose in adults, while in children this amount is even lower. Therefore, the detoxification of flax seeds and in particular their pomaces is important for their maximum use in the food chain **(Bekhit et al., 2018)**.

Contact between endogenous β-glycosidases located in the cell wall with cyanogenic glycosides in the cytoplasm is accelerated mainly by disruption of the cellular structure of flaxseeds **(Mkpong et al., 1990)**. The naturally occurring endogenous enzymes in flaxseed are linustatinase and linamarase **(Fan and Conn, 1985)**. Linustatinase hydrolyzes linustatin to linamarin and neolinustatin to lotaustralin; the monoglycosides are then further hydrolyzed to cyanohydrin and glucose by linamarinase **(Custer et al., 2003)**. Linustatin and neolinustatin are the major glycosides in flaxseed, while the monoglycosides linamarin and lotaustralin are generally found in trace amounts in mature seeds **(Bacala and Barthet, 2007)**.

High levels of cyanogens in flax pomace can cause health problems and thus limit their use in human nutrition **(O'Brien et al., 1992)**. Several studies have been carried out to reduce the cyanogen content of flax seeds. Solvent extraction has been shown to remove cyanogens from flaxseed meal **(Wanasundara et al., 1993; Varga and Diosady, 1994)**. However, this method is demanding, unprofitable and unsuitable for bioproduction. Most strikingly, it causes the loss of beneficial polar compounds, such as lignans **(Yamashita et al., 2007)**. Oven drying has not been shown to reduce cyanogen content **(Feng et al., 2003)**, suggesting their thermostability at high temperature. It has also been found that their content in flaxseed meal has decreased by up to 53% after repeated pelleting **(Feng et al., 2003)**. Other potential methods of removing cyanogens are extrusion **(Wu et al., 2008)**, microwave oven **(Feng et al., 2003)** and repeated soaking **(Yamashita et al., 2007)**, which can reduce 85–90% of the cyanogen content in flax seeds and flax meal.

1.1.3 Importance and use of flax

Flaxseeds are available in two basic varieties: brown and yellow/golden. Both have similar nutritional characteristics and equal numbers of short-chain ω-3 fatty acids. The exception is a type of yellow flax called solin (trade name Linola), which has a completely different oil profile and is very low in ω-3 fatty acids **(Dribnenki et al., 2007)**. Brown flax is better known as an ingredient in paints, varnish, fiber, and cattle feed **(Kozlowska et al., 2008; Faintuch et al., 2011)**. Various edible forms of flax are available in the food market: whole flaxseeds, milled flax, roasted flax, and flax oil. Potential flax utilisation is presented in Figure 1 and discussed bellow.

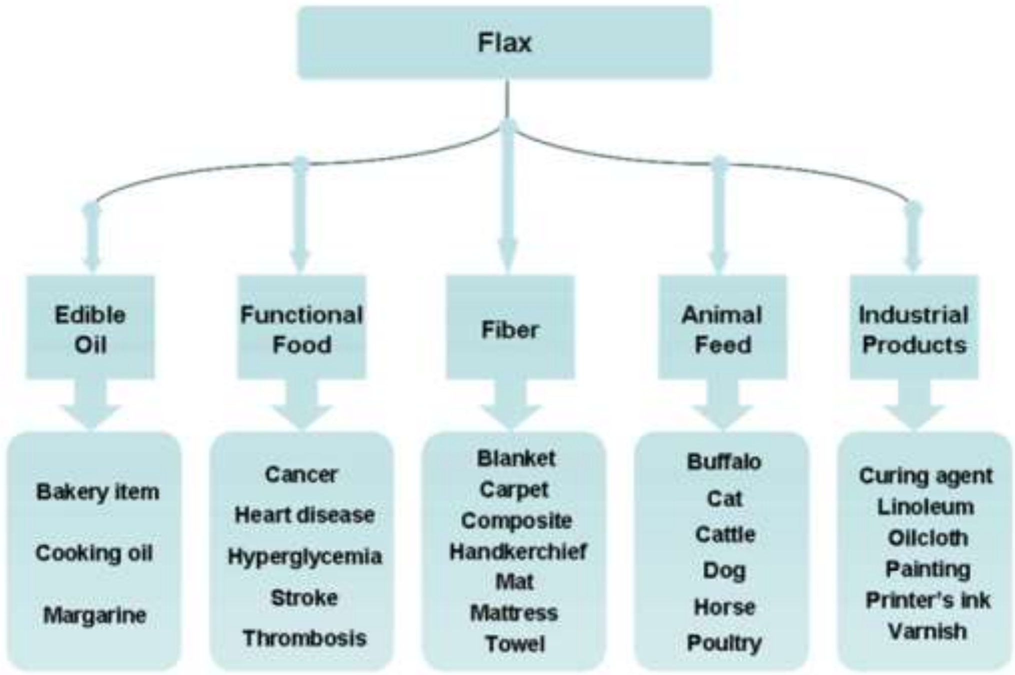

Figure 1: Flax utilistaion **(Jhala and Hall, 2010)**

Flax in Human Consumption

Flax was being used as a food source and natural laxative dating back as far as the ancient Greeks and Egyptians. It was also being used as a food in Asia and Africa **(Berglund, 2002)**. The unique and diverse properties of flax are reviving interest in this crop. In 2005, approximately 200 new food and personal care products were introduced in the US market containing flax or flax ingredients **(Morris, 2007)**, which suggests that flax-based products have the highest growth potentials in functional food industry.

Conventional flax seed, containing a mixture of the fatty acids, is rich in two essential fatty acids, ω-3 alphalinolenic acid (ALA; C18:3) **(Foulk, Akin and Dodd, 2002; Canadian**

Egg Marketing Agency, 2007) and ω-6 linoleic acid (LA; C18:2) **(Bloedon and Szapary, 2004)**. In an average Canadian flax cultivar, ALA comprises about 57% of the total fatty acids in flaxseed, whereas ω-6 comprises about 16%, giving ω- 6/ω-3 ratio of 0.3:1.0. The typical western diet is high in ω-6 and low in ω-3. Current dietary ω-6/ω-3 ratio ranges from 10:1 to 25:1 while recommended ratio is 4:1 to 10:1, especially for pregnant women and infants **(Scientific Review Committee, 1990)**. Consuming flax or other food rich in alpha linolenic acid like fish oil, ω-3 enriched eggs, increases ω-3 intake, which improve ω-6/ ω-3 ratio.

Flax is the richest source of ALA, a precursor for the synthesis of very long chain polyunsaturated fatty acids (VLCPUFAs), eicosapentaenoic acid (EPA, C20:50) **(Bhathena et al., 2002; Fitzpatrick, 2007)** and docosahexaenoic acid (DHA, C22:6) **(Blumenthal et al., 2000; Berglund, D.R., 2002)**. Metabolism of ALA in animals by a series of alternating desaturations and elongations, converts it into VLCPUFAs, EPA, and DHA. Conversion of ALA to VLCPUFAs in humans is affected by various hormonal changes and dietary factors **(Yamazaki et al., 1992)**. High level of ω-6 fatty acids in food supply interferes with the conversion of ALA to EPA and DHA because ω-3 and ω-6 family compete for the same desaturase enzymes.

The ω-3 fatty acids, particularly DHA, are required for the optimal development of nervous system and maturation of visual acuity (retina) in preterm and term infants (Uauy et al., 1996). EPA and arachidonic acid (AA, C20: 4) **(Bhathena et al., 2002; Fitzpatrick, K., 2007)** are the precursors of eicosanoids and components of mammalian cell membranes, including the prostaglandins, blood clotting, cell signaling and blood pressure regulation **(Kinsella et al., 1990)**. Deficiency in ω-3 increases the chances of diabetes, cancer, arthritis, inflammatory diseases, depression, heart disease, hypertension, memory problems, weight gain and some allergies **(Morris, 2007)**.

In leafy green plants, fatty acids are usually in the form of ALA alone; however, their over all lipid content is very low, so they can not meet the total requirement of ALA alone. Most fish contain only trace amount of ALA, although a few species of fish such as salmon are rich in EPA and DHA **(Nelson and Chamberlain, 1995)**. However, the consumption of fish oil is predicted to continue to decrease because of diminished global fish stocks and heavy metals contamination of oils derived from fish. For vegetarian diets, flax is the richest plant source of ALA.

Flax oil

Flaxseed is the richest plant source of the ω-3 fatty acid, that is α-linolenic acid (ALA) **(Goyal et al., 2016a)**. Flaxseed oil is low in saturated fatty acids (9%), moderate in monosaturated fatty acids (18%), and rich in polyunsaturated fatty acid (73%) **(Cunnane et al., 1993)**. In flaxseed oil, α-linolenic acid is the major fatty acid, ranging from 39 to 60.42% followed by oleic, linoleic, and palmitic and stearic acids, which provides an excellent ω-6:ω-3 fatty acid ratio of approximately 0.3:1 **(Pellizzon et al., 2007)**. Although flaxseed oil is naturally high in antioxidants like tocopherols and beta-carotene, traditional flaxseed oil is easily oxidized after being extracted and purified **(Goyal et al., 2016b)**. The bioavailability of ALA is dependent on the type of flax ingested; ALA has greater bioavailability in oil than in milled seed, and by comparison, the least bioavailability in whole seed form **(Austria et al., 2008)**.

The direct use of unprocessed conventional flax oil in the human diet is limited by product stability. Linseed oil with high ALA is highly susceptible to oxidation and polymerization. While these properties make it suitable for other industrial applications, it limits the direct substitution of flax oil in place of canola or corn oil. The oil properties of flax are so unique that considerable effort is being expended to emulate the fatty acid profile. Modification of soybean oil and canola oil using conventional and molecular approaches to enhance the ALA content and therefore the health benefits and to replace fish oils in the diet are an active area of research **(Scarth and Tang, 2006)**.

Flax for food processing

Recently, flaxseed has been the focus of increased interest in the field of diet and disease research, due to the potential health benefits associated with some of its biologically active components. Flaxseeds have nutritional characteristics and are rich source of ω-3 fatty acid: α-linolenic acid (ALA), short chain polyunsaturated fatty acids (PUFA), soluble, and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside, SDG), proteins, and an array of antioxidants **(Goyal et al., 2014a)**. Its growing popularity is attributed to its imparting numerous health benefits to consumers, including reducing the incidence of cardiovascular diseases, decreased risk of cancer (particularly of the mammary and prostate gland), antiinflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis **(Goyal et al., 2014b)**.

Whole flaxseed products

Flax is a poorly competitive crop with few registered weed control options **(Brook and Cutts, 2014)** and admixed weed seed is a common contaminant of flaxseed. Furthermore, organic flaxseed must be grown on relatively weedfree fields to avoid heavy contamination as the use of herbicides is not compatible with organic production **(Bilalis et al., 2012)**. Flaxseed must be thoroughly cleaned prior to use for food applications. Commercial seed may include admixtures of weed seed and volunteer seed from previous crops **(Canadian Grain Commission, 2014)**. Flaxseed may be cleaned with a colour sorter to remove weed seed or offcoloured seed **(Pearson, 2010)**. It has been also reported that individual flaxseed grains (<5%) may have a darker colour than bulk grain. Selectively removing the darker coloured seed with a colour sorter produced a uniformly coloured product that had superior quality characteristics for further use **(Pizzey, 2002)**. The flat shape of flaxseed can result in inaccurate colour sorting **(Pearson, 2010)**.

Roasted whole flaxseed is cleaned to remove weed seed, damaged seed, and other contaminants. Cleaning should be thorough as contaminating seed is very evident in roasted flaxseed. The resulting clean seed is roasted to produce a product that can suitably be used as a food or condiment. In this product category, yellow seed colour is seen as more aesthetically pleasing and yellow seeded flaxseed is preferred. The use of "golden flaxseed" in the names of such products is common. Milled whole flaxseed is also cleaned to remove weed seed and other contaminants. Milled seed may be heated or sold as a product without prior heating. Without heating it is likely that the flaxseed would harbour bacteria **(Morita, Murayama and Iida, 2003)** and have some trypsin inhibitor **(Bhatty, 1993)** proteins. There is no standard roasting time or temperature known for flaxseed, but standard desolventiser toaster conditions can remove bacterial contamination.

Milled flaxseed has been sold as a product that is typically packaged in an inert gas (typically nitrogen) or under vacuum. Milled flaxseed may also be blended with other nutraceuticals including powdered blueberry and pomegranate or mixed in a high fibre blend with oat bran and chia. Similarly, flaxseed can be milled after sprouting and drying the seed. Fermentation products produced by fermenting crushed and milled flaxseed with probiotic bacterial strains including *Lactobacillus acidophilus*, *L. rhamnosus*, *L. plantarum*, and *L. fermentum strains*, as well as *Bifidobacterium strains* were patented **(Salminen, et al., 2012)**.

Milled flaxseed contains some sucrose and raffinose **(Amarowicz and Shahidi, 1994)** that may be fermented using a variety of probiotic bacteria **(Salminen et al., 2012)**. Fermentation of 10% flaxseed in water produced a suspension that was described as viscous and slimy. Bacterial populations of 108 to 109/mL were achieved with a pH of less than 4.0. Using lower concentrations of flaxseed produced a more acceptable product with a yogurt like consistency.

Whole flaxseed may also be included in animal feed products to modify the lipid composition of animal products by acting as a precursor to omega-3 fatty acids. Typically, flaxseed will be a minor component of the whole ration but there are exceptions. For example, flaxseed has been co-extruded with pea to produce a ration with greater digestibility **(Thacker, Racz and Soita, 2004)**. One commercial product, a 50:50 combination of extruded full-fat flaxseed and peas, is an example of this application of flaxseed. The shelf-life of whole milled flaxseed products can be extended by removing darker coloured flaxseeds with a commercial colour sorter **(Pizzey, 2002)**. However, it has been reported that flaxseed meal has substantial stability without the removal of darker seed **(Malcolmson, Przybylski and Daun, 2000; Przybylski and Daun, 2001)**. The characteristics of flaxseed meal are highly dependent on seed variety. The viscosity produced by addition of whole flaxseed meal to water varies significantly between varieties **(Bhatty, 1993; Diederichsen et al., 2006)**.

A study in Europe indicates that the consumption of flax oil for 12 weeks (one tablespoon, providing 8 g ALA.day^{-1}) in daily diet lowered blood pressure significantly in middle aged men with high blood cholesterol levels **(Paschos et al., 2007)**. A role of the flax oil in preventing thrombosis has been reported in a study by 40% increase in the activated protein ratio in a population who consumed flax oil diet for six weeks **(Allman-Farinelli et al., 1999)**. In a study of 50 men with high blood cholesterol levels who consumed one tablespoon of flax oil daily for 12 weeks reduced 48% C-reactive protein (CRP) and 32% serum amyloid A (SAA) levels **(Paschos et al., 2005)**.

Defatted and partially defatted flaxseed flour products

As there are many processes used for flaxseed extraction, the flaxseed meal byproduct is not uniform. For example, pressing flaxseed produces an expeller cake that contains less that 10% oil **(Savoire, Lanoisellé and Vorobiev, 2013)** while hexane extraction of the seed cake may follow producing a cake with very little oil. The latter, solvent extracted product, has very different properties than pressed products. Both the press cake and solvent extracted press cake contain several compounds including lignan, mucilage, orbitides, cyanogenic glycosides, and cadmium. Lignan content is cultivar dependent with SDG contents varying from 6.1 to 13.3 $mg.g^{-1}$ seed dry matter **(Johnsson et al., 2000)** and 11.7–22.7 $mg.kg^{-1}$ in defatted flaxseed flour of the same cultivars. Partially defatted flaxseed meal is an excellent bakery ingredient and may be used as a gluten replacement in gluten free cooking. Flaxseed also contains trypsin inhibitors and condensed tannins that may impact the quality of flaxseed flour products. **Russo and Reggiani (2013)** investigated the levels of antinutrients in hexane-extracted flour from seven flax cultivars. They reported significant differences in phytic acid, condensed tannins, and trypsin inhibitor but not cyanogenic glycosides among the cultivars tested. This was, in part, in agreement with **Bhatty (1993)** who reported that the trypsin inhibitor levels differed between flax varieties but also found differences between the cyanogenic glycoside content of the same cultivars. Despite its content of linolenic acid, flaxseed flour arising from cold pressing has excellent oxidative stability **(Aladedunye, Sosinska and Przybylski, 2013)**. When petroleum ether defatted flaxseed meal was mixed with commercially purchased flaxseed oil it was found that the antioxidant system of the meal confered stability to the oil **(Barthet, Klensporf-Pawlik and Przybylski, 2014)**.

Enriched flaxseed lignan-bearing products

The flaxseed lignan occurs predominantly or possibly exclusively in seed hull fractions. Dry fractionation of flaxseed to produce hull-enriched fractions may be accomplished using several milling steps. **Oomah, Mazza and Kenaschuk (1996)** utilized a tangential abrasive dehulling device to abrade hull from seven flaxseed cultivars and found that hullability was a function of genotype and dehulling conditions. They did not measure the amount of lignan in the hulled materials, as the method used for dehulling did not allow the recovery of the hull fraction. In this study hullability correlated positively with seed size in the cultivars tested. **Cui and Han (2006)** used an abrasive mill with a different configuration to dehull flax and were able to recover a hull rich fraction that had 2 to 10 times the lignan content of whole flaxseed.

Muir and Westcott (1998) devised a process to extract and purify lignans from whole flaxseed using alcoholic solvents followed by base hydrolysis. The product of these separations was a highly enriched preparation of secoisolariciresinol diglucoside (SDG). Later **Westcott and Paton (2001)** developed a simplified process for isolation of a flaxseed fraction enriched in lignan complex. which may be included with foodstuffs in the form of a complex. Normal digestion and metabolism of the complex releases lignan, thus, the complex has been the subject of considerable research **(Katare et al., 2012; Landete, 2012)**.

Products of wet dehulling

Dehulling is also possible following the extraction of seed gums from whole seed with water. After soaking whole seed, the hull may be removed by placing it in a Waring blender with a modified blade (i.e., a blade covered with rubber tubing). The high shear conditions afforded a wet hull fraction mixed with “kernels”. The two materials are readily separated from the mixture by settling as the hull components settle slowly compared to the kernels **(Kadivar, 2001)**.

Products of sprouted flaxseed

The natural early growth of the seed, germination, leads to shedding of the seed hull before the seeds reserves are consumed **(Stewart, 2006; Föglein, 2011)**. Hull-free flax sprouts may be used as a food supplement for fishmeal, fish oil, algae, or dietary supplements **(Stewart, 2006)**. This product may be powdered then combined with other ingredients, which include talc and clay. Food products can also be produced from mucilage-free flax sprouts **(Föglein, 2011)**. Flaxseeds treated with enzymes (aqueous pectinase, protease, and cellulose) were substantially freed of mucilage. The mucilage depleted seed was then sterilized to reduce microbial populations. The live seed could then be sprouted to obtain mucilage-free flax sprouts. Caution must be used in the production of sprouted flaxseed products as it has been established that cyanogenic acid monoglucosides linamarin and lotaustralin accumulate after two days of imbibition **(Niedźwiedź-Siegeń, 1998)**.

Flax fiber and its uses

The latest update of the European Commission’s (EC) Bioeconomy Strategy sheds light on the strategic role of bio-based products and services in the transition towards a postfossil carbon economy, bringing both innovative and sustainable solutions to global challenges such as climate change, land- and ecosystem degradation. Because it allows decoupling the material and in particular, the chemical sector from the use of fossil carbon, bio-based materials are a growing and encouraged market throughout Europe **(EC, 2018b)**. In France, for example, the national Bioeconomy Action Plan suggests using bio-based materials for the construction of the Olympic Village 2024 **(Embassy of France in Washington, 2018)**, among others.

Flax (*Linum usitatissimum*) has been long used as a source of textile fibers. Lately, it has also been used to meet technical applications such as reinforcement for composite materials. In fact, natural-fiber reinforced materials are increasingly being used as a substitute for glass fiber reinforced composites, particularly in the automotive sector **(Yan et al., 2014; Deng and Tian, 2015)**, since it allows a weight reduction of parts of ca. 5% **(Le Duigou and Baley, 2014)**, among other benefits. Amongst the different natural fibers being used, flax represented 50% of the market share for composites in 2012 **(Barth and Carus, 2015)**.

Worldwide, Europe accounts for 70% of the world's flax production, with the French Normandy region responsible for 85% of the European production **(C.E.L.C., 2010a; FAOSTAT, 2017)**, making France the world leader of flax fiber production. This reflects the suitable agronomic conditions provided in the North of France (humid climate and nutrient rich soils) for the cultivation of flax, combined with a long-established know-how for cultivating and supplying this crop for the flax seed and fiber market. At the European level, there are about 140 flax fiber-processing plants and France has the installed capacity to carry out all stages of the supply chain **(C.E.L.C., 2010a)** i.e. the cultivation stages up to the final weaving into technical or textile fabric.

C.E.L.C. (2010a) states that the cultivation of 1 ha of flax fiber contributes to stock 3.7 tonnes of CO_2 (below-ground carbon). Moreover, most co-products generated through the flax fiber transformation stages are re-circulated into the economy and valorized as new products **(C.E.L.C., 2010b)**. The environmental performance of flax fiber reinforced composites has been assessed in previous Life Cycle Assessment (LCA) studies **(Le Duigou, Davies and Balei, 2011; Bensadoun et al., 2016; Bachmann et al., 2017)**. However, these studies do not focus on the production of the flax fiber technical textile per se and, therefore, lack detailed information on the Life Cycle Inventory (LCI). Additionally, co-products are handled by using economic allocation techniques and their fate is little discussed or specified.

Flax thus appears as a potentially important feedstock to a Europe aiming to deploy a sustainable bioeconomy. However, the full consequences induced using flax as a source of fiber to replace glass fibers has been little studied **(Gomez-Campos et al., 2021)**.

Current work on LCAs assessing the environmental impact of flax fibers as reinforcement on composite materials is limited and what is available either does not address the whole supply chain of flax fiber transformation (from cultivation to weaving into a technical textile) or lacks transparent information on the LCI used. **Le Duigou et al. (2011)** presented LCI data up until the combing process and used mass allocation to artificially attribute parts of the impacts to the studied product only. On the other hand, **Deng and Tian (2015)** did follow a consequential approach, but accounted only for seeds, short fibers, shives and flax tow as co-products; whereas in this work every co-product emerging in the supply chain were accounted for, along with consequences of demanding constrained resources (e.g., land use changes).

Use in animal diet

Flax is integrated into animal rations in several forms; whole seed, oil supplements, hulls, or as meal. Meal, known as linseed cake in Europe and Asia, is the residue after the extraction of oil from seeds. This valuable feed product can be used to supplement the diets of both ruminants and non-ruminants **(Jhala and Hall, 2010)**.

The quantity of hull in flax seed meal is about 38%, twice the level in canola or soybean meals **(Agriculture and Agri-Food Canada, 1997)**. The fine fraction obtained as a byproduct of dehulling (a process of preparing flaxseed for value added industrial products) could be a potential ingredient in pet food, whereas the medium and mix fractions can be blended into poultry feed formulations **(Oomah, Kenaschuk and Mazza, 1996)**. Flax seed oil is also used in mixed pet diets, including dogs, cats, and horses. The essential fatty acids (ALA and LA) present in flax seed contribute to a lustrous coat, help prevent dry skin and dandruff, and help in reducing digestive and skin problems in animals **(Jhala and Hall, 2010)**.

The ω-3 enriched eggs are produced by increasing ground flax seed to 10–20% of the diet of laying hens. Eggs produced from this diet formula would be ten times higher in ω-3 fatty acids than conventional eggs **(Canadian Egg Marketing Agency, 2007)**. A single ω-3 enriched egg provides half of the optimal daily intake of ALA and about one quarter of EPA and DHA **(de Lorgeril et al., 1999)**.

Feeding n-3 PUFA to pigs, using linseed, improves pork nutritional quality. A meta-analysis involving 1006 pigs reported in 24 publications was carried out to assess the effects of dietary linseed on alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) content in muscle and adipose tissue. Data showed positive effects of n-3 PUFA on muscle fatty acid composition: ALA + 137%, EPA + 188%, DPA + 51% and DHA + 12%. Same results were observed in adipose tissue: ALA + 297%, EPA + 149%, DPA + 88% and DHA + 18%. A positive correlation between dietary treatment and ALA and EPA content in muscle and adipose tissue was observed. A significant association between DPA and DHA and liveweight inmusclewas observed. Feeding linseed to pig improved the nutritional pork quality, raising the n-3 PUFA content in muscle and adipose tissue **(Corino et al., 2014)**.

Feeding flax preparation LinPro to cows at 23 g.kg^{-1} and 47 g.kg^{-1} of diet successfully transferred dietary ALA into milk with marked increases in the n-3 to n-6 FA ratio which improved the health-related quality of the milk to its human consumers. Increased diet net energy for lactation density in both LinPro fed groups indicated more efficient use of dietary energy for milk production which, at least partially, was responsible for the overall increase in milk production as the dietary level of LinPro increased. There was also an improvement in general health (i.e., reduced somatic cells count and culling for mastitis) for cows fed with flaxseed preparation LinPro **(Swanepoel and Robinson, 2019)**.

Consumption of the PUFA of the n-3 family offers a wide range of human health benefits including improved cardiovascular health and cognitive function. Eggs are probably the first line of n-3 enriched food products launched successfully in the marketplace. Flaxseed, owing to its high fat (>38%) and ALA (>50%) contents along with other nutritional properties (e.g., metabolizable energy and protein), is the most common feed ingredient explored for egg n-3 fatty acid enrichment. Its increase in laying hen diets by as little as 1% resulted into an increase in n-3 fatty acids deposition by 40 mg per egg. Overall, human consumption of 2 eggs from hens fed with 10% flax can provide over 440 mg ALA and 170 mg of long-chain n-3 fatty acids. Chicken eggs are one of the most popular and affordable food items for all cultures around the world. Use of flax in layer hen diets and producing eggs rich in n-3 fatty acids is one of the natural, successful, and sustainable way to meet the human requirement of n-3 fatty acids **(Cherian, 2017)**.

Supplementation of false flax (*Camelina sativa*) pomace in dairy goat diets increased the concentration of PUFA and c9t11-conjugated linoleic acid (CLA) in milk. Kefir made from milk of goats fed a CS cake supplement, in comparison to that produced from milk of goats fed basal diet, shows a significant increased contentof bioactive components (PUFA, including CLA) in the fat fraction. No differences were observed in the basic chemical composition or in taste, aroma, or consistency betweenkefir produced from milk of nanny goats from both feeding groups **(Pikul et al., 2014)**.

Overall, the dietetic enrichment with flax seed pomace, which simultaneously overexpresses crucial enzymes in the flavonoid biosynthesis pathway (chalcone synthase, chalcone isomerase and dihydroflavonol reductase) and is rich in flavonoids (quercetin, kaempferol), phenolic acids (caffeic, ferulic, p-coumaric), anthocyanins and secoisolariciresinol diglucoside may be a solution to several the health issues resulted from improper diet in both humans and animals **(Matusiewicz et al., 2015)**.

1.2 Pumpkin (*Cucurbita* sp.)

1.2.1 Characteristic of pumpkin

Pumpkin belongs to the genus *Cucurbita* and family *Cucurbitaceae* **(Ningthoujam, Prasad and Palmei, 2018)**. *Cucurbitaceae* play a fundamental role in the economy and culture of various societies. Many of the species in this family were the first plants domesticated by humans, being used as food or medicine **(Lira and Caballero, 2002)**. Although currently the highest production is found in Asian countries **(FAOSTAT, 2017)**, the genus *Cucurbita* is native to America. Before the arrival of the Spaniards in 1492, several species of pumpkins were pillars of pre-Hispanic agriculture and were traditionally cultivated together with corn (*Zea mays*) and beans (*Phaseolus vulgaris*). In this way, the corn served as a support for the beans and provided shade for the pumpkin; meanwhile, beans fixed nitrogen in the soil, while pumpkin prevented loss of soil moisture and weed growth **(OECD, 2016)**. In Mexico, this sowing system is known as “milpa” **(Ferriol and Picó, 2008)**. After the arrival of the Spanish conquerors in American lands, the existence of pumpkins in other continents was reported and the evidence was published between 1503 and 1508 (only 11 and 16 years after the arrival of Christopher Columbus to the New World) as the first image of a *Cucurbita* outside of America in the book Grandes Heures d’Anne de Bretagne, in an illustration made by Jean Bourdichon in Touraine, France and corresponding to a *Cucurbita pepo*. subsp. *texana*. However, the arrival of domesticated species of *Cucurbita* to Europe took place a decade later **(Paris et al., 2006)** where they continued to be harvested.

The *Cucurbitaceae* family is one of the important ones among vascular plants; it includes 118 genera and 825 species. Mexico is the most important center of diversity where 34 genera (five of them endemic) and 142 species are found **(Lira and Caballero, 2002)**. Among the *Cucurbitaceae* family, the genus *Cucurbita* is found, in which 9 species are described. From these, *Cucurbita argyrosperma*, *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo* are harvested and have importance in terms of agricultural production **(OECD, 2016)**, while *Cucurbita okeechobeensis*, a wild species, is practically extinct. The decrease and extinction of wild species of *Cucurbita* is directly related to the decrease of the megafauna responsible for the seed dispersal and the loss of habitat. The wild and domesticated species of *Cucurbita* present notable differences. Most domesticated subspecies show a greater variety of colors, shapes, and sizes than wild species. In addition, in the cultivated species, domestication has favored germination to be more uniform, and the size of fruits and seeds has increased; however, resistance to diseases and pests has been reduced **(Kates, 2019)**.

The different species of *Cucurbita* are cultivated for their seeds and fruits, which are mainly used as food. In addition to the fruit, the seeds are probably one of the most important objectives for which some pumpkin species are harvested. Once they are collected, cleaned, and baked, the seeds can be consumed directly as a “snack”. They are also ground to make a kind of pasta, with which various dishes are prepared **(OECD, 2016)**. In some cases, stems, leaves, and flowers are also consumed. Furthermore, the uses that have been given to pumpkins are very diverse, for example, it has been reported that the peels have been used as containers, mainly by semi-nomadic people before the pre-ceramic era, or, due to their saponin content, the pulp is used to make soaps **(Kates et al., 2019)**.

Generally pumpkin seeds are by-product in the food industry. Like other members of Cucurbitaceae, pumpkin fruits bear numerous seeds. Pumpkin are variable in size, shape, colour, and weight. Depending upon the polyphenolic pigments present in it, the colour of pumpkins ranges from golden-yellow to orange flesh, having a weigh of 4–6 kg with the largest capable of reaching a weight of over 25 kg. They have a moderately hard flesh with a thick edible flesh below and a central cavity containing numerous small, off-white-colored seeds interspersed in a net-like structure. The seed content of pumpkin fruit varies from 3.52% to 4.27% **(Ningthoujam, Prasad and Palmei, 2018)**.

1.2.2 Nutritional composition of pumpkin

Pumpkin seeds have high nutritional value, that provides good quality oil, and excellent source of protein and have pharmacological activities such as antidiabetic, antifungal, antibacterial and antiinflammation activities, and antioxidant effects. They have historically been used to produce oil, fortify breads, consumed as a snack or even for medicinal purposes. The pumpkin seed has many health benefits and are consider as nutritional powerhouses, with a wide variety of nutrients **(Ningthoujam, Prasad and Palmei, 2018)**.

Basic nutritional composition of of the pulp, peel, and seeds of the three most abudant pumpkin species are presented in Table 2.

Table 2: Chemical composition of the pulp, peel, and seeds (g.kg^{-1} of raw material) of pumpkin species **(Kim Young et al., 2012)**

Chemical Composition	**Part**	***Cucurbita pepo***	***Cucurbita moschata***	***Cucurbita maxima***
Carbohydrates	Peel	43.76	96.29	206.78
	Pulp	26.23	43.39	133.53
	Seed	122.20	140.19	129.08
Protein	Peel	9.25	11.30	16.54
	Pulp	2.08	3.05	11.31
	Seed	308.83	298.11	274.85
Fat	Peel	4.71	6.59	8.59
	Pulp	0.55	0.89	4.20
	Seed	439.88	456.76	524.34
Fiber	Peel	12.28	34.28	22.35
	Pulp	3.72	7.41	10.88
	Seed	148.42	108.51	161.54
Water content	Peel	935.98	871.86	756.79
	Pulp	967.70	942.31	840.43
	Seed	74.06	51.79	27.51

With the global production of 27 million metric tons annually **(FAO, 2018)**, pumpkin is one of the well-studied disease-preventing vegetables **(Adams et al., 2011; Shim et al., 2014; Bronzi et al., 2016; Naziri, Mitić and Tsimidou, 2016; Siano et al., 2016)**. Currently, the interest of public and health professionals towards the importance of functional foods in the prevention of diseases is gaining its grounds **(Shim et al., 2014; Bardaa et al., 2016; Wang and Zhu, 2016; Balbino et al., 2019; Fornara et al., 2019; Plat et al., 2019; Yang et al., 2019)**. Pumpkin seeds are densely packed with valuable functional nutrients. While nutrients in the pumpkin seeds serve as the principal metabolites that sustain life, functional ingredients in the seeds play key roles in disease prevention and health promotion in human beings **(Adams et al., 2011; Rodríguez-Miranda et al., 2012; Pham et al., 2017)**.

Principal fatty acids in pumpkin seed oil (PSO) are linoleic, oleic, stearic, and palmitic that cover more than 95% of total fatty acids and about 75% of which are unsaturated fatty acids (UFAs) **(Benalia et al., 2015; Siano et al., 2016; Bialek et al., 2017; Meru et al., 2018)**. Small concentrations of arachidic and linolenic acid have also been reported **(Adams et al., 2011; Montesano et al., 2018; Balbino et al., 2019; Geranpour, Emam-Djomeh and Asadi, 2019)**. The unsaturated fatty acids have been extensively studied due to their protective effect against cardiovascular diseases **(Gossell-Williams et al., 2008; Plat et al., 2019)**. They are important for healthy growth and development of brain and nervous system, respectively; also, they are reported to have health benefits in the amelioration of coronary heart diseases, hypertension and arthritis **(Meru et al., 2018; Amin et al., 2019; Plat et al., 2019; Yao et al., 2019)**; not to mention inflammation, autoimmune-related disorders and cancer **(Dakeng et al., 2012; Rios et al., 2012; Li and Leung, 2014; Chari et al., 2018)**. Moreover, only two fatty acids are known to be essential for humans, linoleic and alpha-linolenic acids, because they cannot be synthesized in the human body and must, therefore, be supplied through diet.

Pumpkin seed is high in crude protein, roughly 35%, and this translates to a significant and different number of amino acids **(Jafari et al., 2012)**. Amino acids play important roles both as building units of proteins and as intermediates in metabolism. The dietary supply of adequate quantity and quality essential amino acids is equally important for physiological functions in human body **(Cai et al., 2003; Rezig et al., 2013)**. Studies show that protein isolates from pumpkin seed resemble those of soybean with high values of bioavailability of amino acids **(Nwokolo and Sim, 1987; Rezig et al., 2013)**. In fact, **(Rezig et al., 2013)** clarified that the globulin’s structure of pumpkin seeds’ protein is analogous to that of legume seeds. This poses an important note because this nutritional similarity may provide an approval of the pumpkin seed protein as a reliable ingredient in formulating nutritious food recipes, hence ameliorating the damaging effects linked to protein malnutrition facing the susceptible communities. Furthermore, protein isolates of pumpkin seeds have promising antioxidative and chelating properties **(Nkosi, Opoku and Terblanche, 2005; 2006; Sarkar et al., 2007)**.

The pumpkin seeds possess a significant amount of valuable minerals as well. The seeds are rich in potassium (K) and relatively lower in sodium (Na), high in calcium (Ca), manganese (Mn), phosphorus (P), and magnesium (Mg). Pumpkin seeds are also good source of trace elements such as zinc (Zn), iron (Fe), not to mention copper (Cu). Minerals such as Zn, Cu, Mn, and Fe possess antioxidant potential hence serve as cofactors of vital antioxidation-dependant biocatalyst **(Seymen et al., 2016; Datta et al., 2019)**. Similarly, the low sodium and high potassium contents in the pumpkin seeds translate to a significant clinical implication for improving cardiovascular health **(Dotto, Maternu and Ndakidemi, 2019)**. Zinc is essential in male reproduction, structural proteins and cellular protection **(Aghaei et al., 2014)**. These mineral concentrations may, therefore, make pumpkin seed a useful ingredient for food fortification, at least for bakery products.

The pumpkin seed oil has been reported as a good source of phenolic compounds thus attracting considerable attention to researchers due to their promising health benefits to humans **(Andjelkovic et al., 2010; Iswaldi et al., 2013; Amessis-Ouchemoukh et al., 2014; Fawzy et al., 2018)**. Phenolic compounds form a wide group of compounds synthesized as the secondary metabolic products in plants **(Iswaldi et al., 2013; Xiang et al., 2019)** possessing key antioxidant properties **(Adams et al., 2011; Rodríguez-Miranda et al., 2012; Pham et al., 2017)**. Thanks to the presence of a hydroxyl functional group that possesses radical scavenging ability making it suitable for reducing the risk of some oxidation-induced degenerative diseases **(Fawzy et al., 2018)**. Studies found that dominant phenolic compounds in the pumpkin seeds are tyrosol, vanillin, p-hydroxybenzoic, caffeic, ferulic, and vanillic acids; and some small amounts of luteolin, protocatechuic, trans-*p*-coumaric and syringic acids. Nevertheless, the direct antioxidizing potential could be compromised by their low bioavailability. This is because phenolics are susceptible to metabolic transformation to form complexes and some other simple compounds. These complexes are less effective than the parent compounds, due to blocking of the phenolic hydroxyl groups responsible for its antioxidizing role **(Peričin et al., 2009; Andjelkovic et al., 2010; De la Rosa et al., 2019)**. Additionally, the lower aqueous solubility of the phenolics is reportedly contributing to their limited bioavailability **(Iswaldi et al., 2013; Bulut et al., 2019; De la Rosa et al., 2019; Yao et al., 2019)**. This problem could be solved by micro- or nano-encapsulation of the PSO to impede its functional loss. Encapsulation of these bioactives is believed to keep their bioactivities against oxidation, metabolic influences and other destructive reactions within the digestive system and cellular components. This effective delivery system is not only limited to the phenolics but UFAs, tocopherols and other phytochemicals as well **(Dotto and Chacha, 2020)**.

Pumpkin seeds are also a good source of vitamin E. This vitamin in the seeds includes four tocopherol and tocotrienol isomers (α, β, γ and δ) **(Azzi, 2019; Rozanowska et al., 2019)**. Their only isomeric difference lies in the number and position of the methyl groups of the chromanol ring **(Rozanowska et al., 2019; Saito and Yoshida, 2019)**. Nevertheless, only one isomer (d-RRR- α-tocopherol) qualifies the criteria of being a real vitamin E **(Azzi, 2019)**. While sunlight is an effective trigger of synthesis of vitamin E in the human body, yet many plant species are good sources of the vitamin. Pumpkin seed, for example, is rich in tocopherols with γ-tocopherol being the dominant isomer trailed by α- and δ-tocopherols. It also contains small amount of α-tocotrienol, β- and γ-tocotrienols. Tocopherols and tocotrienols in pumpkin seeds are powerful antioxidants with the ability to deactivate highly-active radicals by releasing H^+ ion from its ring **(Bharti et al., 2013; Azzi, 2019)**. In so doing, they keep cell's lipids from peroxidation hence reduced risk of oxidative threats **(Rozanowska et al., 2019; Traber et al., 2019)**. Tocopherols may also serve as prooxidants and decrease some quantity of transition metals in the tissues. The mechanism for the action is, however, highly dependent on the tocopherol levels. Despite the central role of tocopherols in plants being that of antioxidant, non-antioxidant functions have been delineated **(Bharti et al., 2013; Broznić, Jurešić and Milin, 2016; Rupérez et al., 2019; Traber et al., 2019)**. As the γ-tocopherol has been lately reported to be effective in neutralizing peroxynitrite – a potent oxidant with an extensive array of cell injuring effects, more interest on cell signalling-related activities of tocopherols has gained its grounds **(Azzi, 2019; Rozanowska et al., 2019; Saito and Yoshida, 2019)**. Lately, studies have revealed that tocotrienols possess considerable anticholesterolemic potential – unique to tocotrienols, not to mention their neuroprotective, cardioprotective, and antitumor properties **(Bharti et al., 2013; Benalia et al., 2015; Azzi, 2019)**. Tocotrienols share several functional features of isolated tocopherols in vitro and they are measurable in plasma in the human and animal subjects **(Rupérez et al., 2019)**, but so far there is limited information of their bioaccumulation in human tissues.

Pumpkin seeds and PSO are valuable source of phytosterols **(Rabrenović et al., 2014)**. Although there are more than 100 different types of phytosterols identified in plant species, the dominant phytosterols reported in PSO are 7-sterols, contrary to most vegetable oils **(Rabrenović et al., 2014; Tantawy, Elgohary and Kamel, 2018)**. This dominancy is succeeded by sitosterol, 7,22,25-stigmastatrienol, 7-stigmastenol, spinasterol **(Bastić et al., 1997; Li et al., 2020)**, just to mention a few. Phytosterols have been intensively and extensively studied for their lowering effects of blood low-density-lipoprotein cholesterols **(Nakano, Inoue and Murakoshi, 2019; Wang et al., 2019)**, which then translate to a reduced risk of cardiovascular threats. Moreover, several studies have concluded that phytosterols lower the risk of some forms of cancers **(Reaver et al., 2019)**, and ameliorate the treatment of prostate complications **(Gossel-Williams, Davis and O'Connor, 2006; Ren et al., 2012; Alhakamy, Fahmy and ahmed, 2019)**. This level of phytosterols in the pumpkin seed make it a suitable alternative nutraceutical in the management of some non-communicable diseases in human **(Dhariwala and Ravikumar, 2019; Hajhashemi, Rajabi and Mardani, 2019)**.

1.2.3 Importance and use of pumpkin

Pumpkin is a versatile fruit and is used for various food processing applications. Ranging from agricultural purposes to commercial and ornamental sales, pumpkins are grown all over the world for a variety of reasons with versatile uses as cooking from the fleshy shell to the seeds, even the flowers, most parts of which are edible **(Ningthoujam, Prasad and Palmei, 2018)**.

Pumpkin oil

The pumpkin (*Cucurbita* spp.), one of the most popular vegetables consumed in the world, has been recently recognized as a functional food **(Adams et al., 2011; Różyło et al., 2014; AlJahani and Cheikhousman, 2017)**. Pumpkin seeds, generally considered agro-industrial waste, are an extraordinarily rich source of bioactive compounds with interesting nutraceutical properties **(Patel, 2013)**. In recent years, several studies **(Bardaa et al., 2016; Medjakovic et al., 2016; Wang et al., 2017)** have highlighted the health properties of pumpkin seed oil against many diseases, including hypertension, diabetes, and cancer. It also shows antibacterial, antioxidant, and anti-inflammatory properties **(Yadav et al., 2010; Perez Guiterrez, 2016)**. Due to the presence of interesting natural bioactive compounds, such as carotenoids, tocopherols, and sterols, pumpkin-derived products have a wide spectrum of biological activity, proven by in vivo experiments **(Dyshlyuk et al., 2017)**.

Because of the positive health effects, research has been focused particularly on the content and composition of fatty acids (FA) and tocopherols in pumpkin seed oil, while, to a lesser extent, other lipid components, such as sterols, alcohols, and phenol acids, have been studied, as is done with other food matrices to identify specific markers characteristic of the plant varieties **(Romano et al., 2014)**. Among the relevant aspects to be considered when dealing with this vegetable, the beneficial effects of using environmentally friendly natural herbicides **(Cimmino et al., 2012)** must be mentioned, since the content of bioactive compounds could be affected, and there could be possible contamination of this vegetable due to the presence of *Fusarium* spp. microfungi and their secondary metabolites **(Mikušová et al., 2013)**, affecting the content of beneficial compounds of the vegetable itself.

Various cultivars of pumpkin seed oil extracted from various pumpkin sources. **Rezig et al., (2012)** studied the chemical composition and oil properties of seeds of a Tunisian variety of pumpkin, Béjaoui (*C. maxima*).

They found that the major FA were oleic, linoleic, and palmitic acids and that the seed oil was rich was tocopherol, while the sterol marker was sitosterol and the predominant phenolic acid was syringic acid. **Siano et al. (2015)** highlighted that saturated FA (SFA) and monounsaturated FA (MUFA) of C. maxima produced in southern Italy showed similar values (25.20 and 25.54%, respectively), while the polyunsaturated FA (PUFA) content was 48.14%. **Habib et al. (2015)** determined the proximate composition of powdered seed and the lipid composition of the oil of C. maxima collected in Bangladesh. They affirmed that the high degree of unsaturation makes the oil suitable for use as valuable drying agent, and lower free FA content indicates suitability of the oil for consumption as food.

Pumpkin for food processing

A significant number of studies have established a relationship between natural bioactive components of foods and health promotion and disease prevention **(Adams et al., 2011; Shim et al., 2016; Siano et al., 2016)**. The foods that qualify this diet-health relationship are known as functional foods **(Pham et al., 2017).** Plants are natural sources of bioactive compounds and are widely used as functional food ingredients. The pumpkin seeds, like other seeds, are rich in functional components. They are high in vitamin E (tocopherols), carotenoids, provitamins **(Broznić, Jurešić and Milin, 2016)**, pigments, pyrazine, squalene, saponins **(Naziri, Mitić and Tsimidou, 2016)**, phytosterols, triterpenoids, phenolic compounds, and their derivatives **(Abou-Zeid et al., 2018; Aktaş, Uzlaşır and Tunçil, 2018; Acorda, Mangubat and Divina, 2019)**, coumarins, unsaturated fatty acids, flavonoids and proteins **(Dakeng et al., 2012; Aghaei et al., 2014)**. Moreover, pumpkin seeds are good source of magnesium, potassium, phosphorus, as well as other minor minerals such as zinc, manganese, iron, calcium, sodium, and copper **(Koh et al., 2018; Amin et al., 2019)**. Some of these bioactives and minerals act simultaneously at different or identical target sites with the potential to impart physiological benefits, promote well-being and reduce the risk of non-communicable disorders such as tumors **(Dakeng et al., 2012; Chari, Polu and Shenoy, 2018)**, microbial infections **(Kabbashi et al., 2014; Brogan and Mossialos, 2016)**, hyperglycemia and diabetes **(Adams et al., 2011; Naziri, Mitić and Tsimidou, 2016)**, oxidative stress associated complications **(Konoshima et al., 1994; Duncan and Duncan, 1997)**, prostate disorders **(Ren et al., 2012; Alhakamy et al., 2019)** and urinary bladder complications **(Shim et al., 2014; Fornara et al., 2019)**. As evidenced from mentioned literature, pumpkin seeds show potential to be used as both a traditional and functional food ingredient provided further animal and clinical investigations are carried out to establish the respective molecular mechanisms and safety profile **(Dotto and Chacha, 2020)**.

Lipid oxidation in fatty food products presents serious challenge, significantly limiting their shelf-life. One of the possible approaches to deal with it is use of high-barrier or active packaging. Oxidation affects the formation of potentially toxic aldehydes through the degradation of polyunsaturated fatty acids, reducing the nutritive value of food, and leads to significant changes in sensory properties. For fatty food products packing, biopolymer packaging materials may provide good alternative to plastic, due to competitive barrier properties to gases, their natural origin and biodegradability **(Hromiš et al., 2022)**. In their study, composite pumpkin oil pomace and duplex pumpkin oil cake/maize zein films were prepared. Potential protective effects pumpkin oil pomace-based pouches were tested for packing fatty food products, and flaxseed oil was used as a model food system. Results showed that pumpkin oil pomace-based films ensure good oxidative stability and less satisfactory sensory quality of oil, without significance changes in oil composition.

The study of **Atencio et al. (2021)** evaluated the functionalization of industrially-generated pumpkin pomace as a texturizing ingredient. The study showed that heating of pumpkin pomace (85 °C for 30 min) significantly increased storage modulus of the pomace-derived suspensions mainly due to starch gelatinization, protein denaturation and other minor impacts of heating on the nature and components of the suspended particles. A combination of partial pectin removal and high-pressure homogenization at 80 MPa further improved the rheological properties of the pumpkin pomace. The functionalization of a complex plant-based matrix such as pumpkin pomace needs an understanding of both changes in microstructural properties (e.g., particle size reduction) and the role of specific components in the matrix (e.g., starch, protein) that can be targetedly modified by various processing steps. In application, the effect of incorporating pumpkin pomace as texturizing ingredient in a food product could be evaluated in terms of sensory characteristics and consumer acceptability. From industrial point of view, the identified strategies for functionalization could be very useful to industries in the context of valorization of by-products with minimal processing.

Use in animal diet

There is evidence that pumpkins are used traditionally and on a small scale in the feeding of several species of domestic animals such as ruminants **(Lans et al., 2007)** and equines **(Lans et al., 2006; OECD, 2016)**. Research on the use of pumpkins in animal feeding and its productivity benefits are attributed to its protein and fat content in the case of seeds, and carbohydrates, minerals, and vitamins in the case of the fruit **(Achilonu et al., 2017)**.

Regarding the fruit, **Dorantes-Jiménez et al. (2016)** mention that the dry residue of *Cucurbita argyrosperma* (peel and pulp only) has low protein content (<9%) but contains almost 50% of neutral detergent fiber and 40% of acid detergent fiber, which makes it suitable for the formulation of diets for dairy cattle and rabbits. On the other hand, **Crosby-Galván et al. (2018)** mention that the ruminal digestibility of dry matter increases 21% by replacing up to 30% of corn stubble with dry residue of *Cucurbita argyrosperma*; however, the digestibility of neutral detergent fiber decreases 7%, which is attributed to the degradation of nonfibrous carbohydrates such as sugars, which are rapidly fermented. Possibly, the sugar content is one of the reasons why the incorporation of pumpkin improves the palatability of the diet. Pumpkin fruits can also be successfully silage, provided they are mixed with other ingredients with lower moisture content, for example, 20% beet pulp. In this case, beets improve silage characteristics by providing easily fermentable carbohydrates **(Łozicki et al., 2015)**.

To date, studies on the use of pumpkins in ruminant feeding are scarce. In cattle, **Halik et al. (2018)** reported an increase of about 6 kg.day^{-1} of milk by replacing 17% of corn silage with *Cucurbita maxima* silage (20.7 vs. 26.5 kg.day^{-1}, respectively), while in buffalos there was no change in weight gain by including up to 14% of *Cucurbita pepo* silage **(Razzaghzadeh, Amini-jabalkandi and Hashemi, 2007)**.

In laying hens, there were no changes in the laying rate or the quality of the egg using pumpkin seed flour **(Hajati, Hasanabadi and Waldroup, 2011; Martínez et al., 2012)**. In the case of turkeys, the use of 5% of seeds in their diet improved the fertility of the eggs, reduced embryonic death, and increased hatching rate **(Machebe et al., 2013)**.

In pigs, research on the use of pumpkins as food is scarce; however, the report by **Medina-Gonzáles et al. (2019)** indicates that the weight gain of pigs is not a ected when up to 30% of the ration is replaced by *Cucurbita pepo* ferment. However, food consumption was increased by 75%, which affected food conversion.

Besides its use as food, various medicinal properties have been attributed to pumpkins. In this regard, different compounds have been reported, both in seeds and fruit, with different biological activities: antioxidant, antifungal, antiparasitic, antimicrobial, and anti-inflammatory **(Yadav et al., 2010; Achilonu et al., 2018)**. Therefore, the health and consequently the productivity and welfare of livestock can be improved with the addition of pumpkin to the diet. In vitro assays in birds have reported that Cucurbita pepo ethanolic extract is effective against Histomonas meleagridis, Tetratrichomonas gallinarum, and Blastocystis sp. which are protozoans of economic importance in poultry farming; however, it has limited effect in vivo **(Grabensteiner et al., 2008)**. It has also been shown that pumpkin seed lectins have antibiotic action against *Salmonella typhymurium, Salmonella gallinarum, Escherichia coli*, and *Pseudomonas*, so their use could decrease the use of antibiotics. Additionally, the whole fruit has action against the New Castle virus **(Achilonu et al., 2018)**. In pigs, the ferment of *Cucurbita pepo* decreases the frequency of diarrhea leading to a decrease in the mortality and morbidity of piglets **(Medina-Gonzáles et al., 2019)**. In general, the cucurbitacins present in pumpkins have digestive and purgative action due to their bitter compound content **(Montesano et al., 2018)**, which is the reason why pumpkins have most likely been used as antiparasitic agents **(Lans et al., 2007; Yadav et al., 2010; Achilonu et al., 2018)**. Additionally, **Bahramsoltani et al. (2017)** demonstrated that 20% *Cucurbita moschata* peel extract can be used successfully to treat burns due to the high mucilage content which reduces oxidative stress of burned tissue.

Pumpkin seed pomace, a byproduct of pumpkin seed oil processing, is used in ruminant feed as a beneficial protein source. Experiments were conducted by **Li et al. (2021)** to evaluate pumpkin seed pomace as a substitute for soybean meal in the diets of lactating cows based on performance, rumen fermentation, antioxidant function and nitrogen partitioning. The cows were randomly divided into three treatment groups, without and with soybean meal replaced with pumpkin seed pomace and dried distillers' grains with solubles at levels of 50% and 100%, respectively. The diets were iso nitrogenous and contained identical roughage but different proportions of pumpkin seed pomace and dried distillers' grains with solubles. Replacement of soybean meal with pumpkin seed pomace and dried distillers' grains with solubles did not influence rumen degradation, milk performance, rumen fermentation, DM intake or apparent total tract digestibility, and nitrogen partitioning between milk, feces and urine did not differ in the animals fed the three diets. However, compared with a diet containing no pumpkin seed pomace, the total antioxidant capacity and antioxidant enzymes (total superoxide dismutase, glutathione peroxidase and catalase) activities were increased in the animals that received the 50% and 100% pumpkin seed pomace. In contrast, addition of pumpkin seed pomace significantly reduced concentrations of aspartate transaminase, alkaline phosphatase and malondialdehyde in the plasma. These results demonstrate that pumpkin seed pomace can be completely substituted for soybean meal in the diet of dairy cows without any negative impact on milk performance, rumen fermentation or apparent digestibility and that this dietary change improves antioxidant functions and blood parameters in dairy cows, indicating that PSC has the potential for use as a feed source for dairy cows **(Li et al., 2021).**

Partial replacement of soybean meal with pumpkin seed pomace promoted adequate carcass characteristics and is feasible with respect to changes in haemato-chemical parameters of lambs' blood in organic farming. Replacing soybean meal with 10% and 15% of PSP had less favourable effects owing to higher contents of linoleic acid (LA) and a higher LA/alpha-linolenic (ALA) acid ratio in *m. semimembranosus*. Thus, pumpkin seed pomace could be used in lamb feed as a replacement for soybean meal in terms of the haemato-chemical parameters and carcass traits. In future research it is necessary to obtain more levels of replacement with PSP and to research its antioxidant potential in lamb feed **(Antunović et al., 2018)**.

In study of **Klir et al. (2017)** the effect of substituting PSP or extruded linseed (ELS) for soya bean meal in goats' diets on milk yield, milk composition and fatty acids profile of milk fat was observed. Dairy goats were divided into three groups and fed with concentrate mixtures containing soya bean meal ELS or PSC as main protein sources in the trial lasting 75 days. Addition of ELS or PSC did not influence milk yield and milk gross composition in contrast to fatty acid profile compared with control group. Supplementation of ELS resulted in greater branched-chain fatty acids (BCFA) and total n-3 fatty acids compared with control and PSC. Total n-3 fatty acids were accompanied by increased ALA (ALA, and EPA proportions in milk of the ELS group. In contrast, ELS and PSC resulted in lower LA proportions compared with control. Abovementioned resulted in lower LA/ALA ratio with supplementation of ELS compared with control or PSC. The PSC diet decreased total n-6 fatty acids compared with the control. Partially substituted soya bean meal with ELS diets may increase beneficial n-3 fatty acids and BCFA accompanied by lowering LA/ALA ratio and increased C18:0. PSP completely substituted soya bean meal in the diet of dairy goats without any decrease in milk production or sharp changes in fatty acid profile that may have a commercial or a human health relevancy **(Klir et al., 2017)**.

1.3 Current status on the oil seeds market

FAO forecasts for 2020/2021 continue to point to a tense situation in the market for oilseeds and oilseed products, with a renewed increase in production not being sufficient to meet global demand. In the years 2020/2021, the recovery of oil crop production from the previous season reduced harvest, is expected. Soybean and oilseed rape production will pick up, which will offset the decline in world sunflower seed production caused by the weather. Although global supplies of meals and pomaces are set to increase, their global consumption is expected to continue to increase, especially in China. As global demand for oilseed meals and pomaces is expected to exceed production, global stocks are expected to decline globally for the second season in a row and their stocks could fall to a seven-year low **(FAO, 2021)**.

Vegetable oils are expected to recover from global production decline in the previous season, largely supported by an increase in palm kernel and soybean production. The global use of vegetable oils is expanding only slightly in both the food and non-food sectors, which is associated with the ongoing COVID-19 pandemic and recorded high vegetable oil prices. As with oilseed meals and pomaces, stocks are expected to decline to global global lows **(FAO, 2021)**.

In the coming season 2021/2022, preliminary forecasts suggest a slight improvement in the global supply and demand ratio, as well as a slight replenishment of oilseeds and their by-products. Initial crop forecasts point to a likely significant increase in global production of oilseeds and vegetable oils, while an increase in the overall use of oilseed products is also expected, which is a reason to slightly replenish their stocks. However, this outlook is stable due to weather conditions in key growing areas, the development of anti-pandemic measures and vaccination campaigns against COVID-19 and national policy measures that could affect the global market for oilseeds and their by-products such as meals and pomaces. The market for oilseeds is also significantly affected by the admixture of oils into biodiesel, which varies from country to country **(FAO, 2021)**.

1.3.1 Production parameters of flax and pumpkin in the world, EU and in the SR

Flax is an important raw material in the textile, food, pharmaceutical, cosmetic and other industries. It has healing effects on the human body; it is used in various diseases. Seeds that are valuable for the content of omega-3 fatty acids and omega-6 fatty acids, lignans, fiber and vitamins are used for therapeutic purposes **(Meravá, 2020)**.

In Slovak statistics, pumpkin belongs to the group of other oilseeds together with hemp, flaxseed, yellow dye, etc. **(Meravá, 2020)**, while in world statistics according to **FAOSTAT (2021)** its production is evaluated as fruit together with other pumpkin species. As mentioned above, the content of pumpkin seeds is 3.52 to 4.27% of the total weight of the fruit **(Ningthoujam, Prasad and Palmei, 2018)** and thus their production can be at least partially estimated.

Comprehensive statistics of harvested areas, hectare yields and total production in the world and in the EU are shown in **Tables 3** and **4**. Production data from Slovakia are discussed below.

Table 3: Areas harvested, hectar yield and total production of flax seed in the world and EU 27 at 2018 – 2019 according to **FAOSTAT (2021)**

World		
Area harvested (ha)	2018	3149344
	2019	3223531
Yield (t.ha^{-1})	2018	9.448
	2019	9.518
Production (t)	2018	2975473
	2019	3068254
EU 27		
Area harvested (ha)	2018	45770
	2019	39830
Yield (t.ha^{-1})	2018	14654
	2019	16857
Production (t)	2018	67070
	2019	67140

Table 4: Areas harvested, hectar yield and production of pumpkin in the world and EU 27 at 2018 – 2019 according to **FAOSTAT (2021)**

World		
Area harvested (ha)	2018	1526112
	2019	1539023
Yield (t.ha^{-1})	2018	149003
	2019	148801
Production (t)	2018	22739602
	2019	22900826
EU 27		
Area harvested (ha)	2018	65720
	2019	66240
Yield (t.ha^{-1})	2018	316126
	2019	324669
Production (t)	2018	2077580
	2019	2150610

Despite its versatile use in the last four years, the sown areas of flax have been significantly reduced in Slovakia. In 2020, its sown area decreased to the lowest area since 1998 (since then, data on flax cultivation have been published on the website of the Statistical Office of the Slovak Republic). According to the inventory of the Statistical Office of the Slovak Republic sown areas as of 20 May 2020, the sown area for flax was 627 ha. Year-on-year, the sown area of flax decreased by 370 ha (-37.1%). With the assumed average yield per hectare of 1.10 t.ha^{-1}, flax production will be achieved in the volume of 690 t, which is a year-on-year decrease of 115.3 t (-14.3%) **(Meravá, 2020)**.

In the 2019/20 marketing year, flax imports increased by a total of 185 tonnes. Of total imports, flax for sowing imports increased significantly, almost 40-fold. Flax exports fell by 280 tonnes. The main importers of flax to Slovakia in the 2019/20 marketing year were Poland (17.3%), Russia (12.7%) and the Czech Republic (12.5%). Flax exports were mainly to Germany (23.0%), the Netherlands (17.0%), Poland (15.4%) and Italy (13.3%) **(Meravá, 2020)**.

In 2019, other oilseeds were sown on an area 154 ha smaller than in 2018. The area of the harvested area decreased by 622 ha and the production of other oilseeds decreased to a volume smaller by 706 t. Of the other oilseeds, the most important is pumpkin for oil production. Of the total harvested area of other oilseeds, its harvested area is 95.5% and the production has a share of 96.7% of the total other oilseeds production. Pumpkin was sown on 4,016 ha, which is 98.4% of the other oilseeds total sown area **(Meravá, 2020)**.

1.4 Poultry nutrition and fattening

1.4.1 Technology of broiler chickens fattening

Fattening of broiler chickens lasts up to the age of 38 to 42 days, live weight is around 2 kg, feed conversion 1.8 kg, mortality up to 4% and yield about 75% **(Brouček, Benková and Šoch, 2011)**.

Hui and Guerrero-Legarreta (2010) state that comfort, health, and production efficiency are decisive factors influencing broiler fattening. In the EU, broilers are usually kept on litter. Standard halls in Europe are windowless and ventilated. After each shift, the used soiled bedding is removed at once and the whole hall is cleaned and disinfected. Subsequently, a new bedding will be spread on the surface at a height of about 40 cm. The halls are open from the side, controlled automatically by a curtain system, and natural ventilation thus ensures a reduction in humidity and gas content. Straw, wood shavings, peat or paper are used as bedding.

The choice of bedding material is influenced by the local economy and material availability. Litter should ensure good moisture absorption, biodegradation, chickens comfort, low dust, absence of contaminants and continuous availability from a biologically safe source. Softwood shavings must be spread evenly, with a height of 8 to 10 cm. If the floor temperature is adequate (28 to 30 °C), a height is possible reduce bedding (if bedside disposal costs are an issue). Concrete floors take precedence over clay floors because they are washable and allow more effective control of the bedding **(Aviagen, 2009)**.

The chickens will get used to the drinkers and feeders in the first days, which will be available throughout the fattening period. Therefore, there must be a sufficient number of feeders and drinkers in the halls. The stress of chickens is also negatively affected by the removal or adding new items to the hall during the fattening period **(Brouček, Benková and Šoch, 2011)**.

Broiler chickens show specific feeding behaviors. At the same time, it is an important characteristic, as they spend 30–50% of their time feeding. They require 14 to 15 thousand bites on feed or other objects and raking every day. Disabling these activities leads to behavioral disorders such as to each other. The chicken can eat right after hatching, but it does not know what kind of feed, as it has only been genetically encoded the size and mechanical properties of the feed. This is learned gradually, and over time, the intake of inedible particles decreases. It is similar with water intake. Rather, the chicken attracts with its physical properties, such as bubbles, dirt particles and luster. After wetting the beak, the drinking reflex is manifested, i. j. head lift and swallowing. Therefore, it is important that chickens have good access to feeders and drinkers and limited access to inedible objects from hatching. Chickens usually receive enough food to cover their energy needs as well as other nutrients. They receive food in small amounts, but often. After fasting for several hours, they tend to take in more food. Synchronized feed intake is also significant in broiler chickens and is more pronounced than hunger. This means that each chicken usually adapts to feed intake according to the whole group **(Tůmová, 2012)**.

Implementing a lighting program should be simple. Successful implementing complex lighting programs can be difficult. Recommendations related to lighting are subject to local laws, which should be considered before starting the program. The lighting program, used by many broiler fatteners, provides in essentially continuous lighting. This system consists of a long period continuous light, followed by a short period of darkness of 30 to 60 minutes. This short period allows the chickens to get used to the darkness for case of power failure. In the initial stages of growth to seven days of age, all programs should light provide a longer time of day such as 23 hours of light and one hour of darkness. The aim is to ensure a good feed intake by the chickens. Too early shortening the length of the day reduces feeding activity and reduces body weight in on the seventh day **(Aviagen, 2009)**.

The utilization of the feed is closely linked to the growth of chickens. Feed utilization can also be improved by a reduced photoperiod, which reduces feed conversion. During the dark phase, feed losses by being thrown out of the feeders are reduced. In addition to nutrition and feeding techniques, the utilization of the feed is also given by the shape of the growth curve. Broiler chickens, which have slowed down their growth due to the reduction in the day-light length, are entering a period of intensive growth with lower weight, which will be reflected in better feed utilization **(Brouček, Benková and Šoch, 2011)**.

1.4.2 Basic nutritional components of feed

Energy

Broiler chickens need energy for tissue growth and activity. Carbohydrate sources, e.g. Maize and wheat, as well as various fats and oils, are the main source of energy in poultry feed. The energy value in nutrition is given in megajoules ($MJ.kg^{-1}$) or kilocalories ($kcal.kg^{-1}$) of metabolizable energy (ME) **(Aviagen, 2009)**.

Protein

Protein components of feed, e.g. The proteins contained in cereals and soybeans are complex substances that are broken down into amino acids by digestion. These amino acids are absorbed and converted into the body's proteins, which are used in the construction of body tissues, e.g., muscles, nerves, skin and feathers. The amount of crude protein in the diet does not indicate the quality of the protein in the feed ingredients. The nutritional quality of proteins depends on the amount, balance, and digestibility of essential amino acids in the feed mixture. Ross 308 broiler chickens are particularly sensitive to the amount of digestible amino acids in the diet. The administration of a balanced feed mixture with a high amount of digestible amino acids has a positive effect on growth, thus also achieving higher feeding efficiency and profitability **(Aviagen, 2009)**.

Macroelements

Ensuring enough minerals in an appropriate ratio is important for the high performance of broiler chickens. The most important macroelements in broiler chicken nutrition include calcium, phosphorus, sodium, potassium, and chlorine. Calcium significantly affects growth, feed efficiency, bone development, runner length, nerve function and the immune system. It should be given in sufficient quantities throughout fattening. Phosphorus is needed for the growth and structure of the skeleton. Sodium, potassium, and chlorine are generally required for metabolic activity. Their deficiency can adversely affect feed intake, growth and blood pH. However, their excessive intake can cause increased water consumption and consequently inadequate bedding quality **(Aviagen, 2009)**.

Trace elements and vitamins

Trace elements and vitamins are needed for all metabolic functions. Their intake depends on the composition of the compound feed, the preparation of the feed and the geographical conditions. Due to the different proportions of these substances contained in different cereals, it is necessary to adapt their administration **(Aviagen, 2009)**.

Energy

Enzymes are currently often applied to poultry feeds to increase the digestibility of the individual feed ingredients **(Aviagen, 2009)**. The commercial use of carbohydrates (feed enzymes) in poultry nutrition began in the late 1980s and early 1990s due to their ability to regulate digestion and various metabolic problems associated with high-fiber feed. Enzymes are used to balance the adverse effects of non-starch polysaccharides on intestinal health and poultry production **(Aftab and Bedford, 2018)**.

1.4.3 Use of antibiotics in broiler chicken production

Over the past 50 years, the use of antibiotics combined with strict biosecurity and hygiene measures has helped the poultry industry to grow by preventing the negative impacts of many avian diseases **(Bermudez, 2003)**. Even as biosecurity may be sufficient, vaccination can also be used as an additional measure. A vaccine helps the immune system by preparing it against certain pathogens such as viruses or bacteria to which it may be exposed in the future. Vaccination protocols and the type of vaccine used vary from country to country and from farm to farm. Many factors can influence the choice of vaccination method such as species, place, number of manpower, type of production, and production cycle. The choice of vaccination method also depends on general health status of poultry, maternal immunity, and vaccine costs. Livestock vaccination against specific diseases is compulsory (e.g., Newcastle disease) in many countries (Belgium, Netherlands, Germany), while in other such as France only long-lived poultry (laying and breeding) are vaccinated **(Rauw et al., 2009)**.

Antibiotics are not effective against fungal and viral pathogens. They only treat infectious diseases whose causative agents are bacteria. In general, antibiotics are used in phytosanitary treatments, fish farming, animal feed, and human or veterinary medicine where they can be used as a preventive or curative treatment. Antibiotics are classified according to their chemical family, mode of action and the species of bacteria on which they act. Bactericidal antibiotics kill bacteria and bacteriostatics weaken them by inhibiting their proliferation and facilitating their phagocytosis by the immune system. Thus, mortality rate decreases because animals become more resistant **(Mehdi et al., 2018)**.

In intensive poultry farming, especially in North America, antibiotics such as tetracycline, bacitracin, tylosin, salinomycin, virginiamycin and bambermycin are often used **(Diarra and Malouin, 2014)**. In the United States, tetracyclines represent more than two-thirds of antimicrobials administered to animals **(Gonzalez Ronquillo and Angeles Hernandez, 2017)**, while in European Union (EU) they represent only 37% **(Carvalho and Santos, 2016)**. In 2015, the overall sales of veterinary antimicrobial agent were 8,361 t in EU **(ESVAC, 2017)**. This figure is calculated without counting growth promoters in animal production **(Kümmerer, 2009)**. The use of antibiotics as growth factors is not allowed in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) participating countries **(ESVAC, 2017)**. In 2014, 1.5 million kg of active antimicrobial ingredients were distributed for use in animals in Canada, up 5% from 2013. For antimicrobials distributed, 99% were for farm animals and less than 1% was for pets. In 2014, 81% of the antimicrobials used in Canada on broiler farms were for prevention purposes. In the feed, 84% of these antimicrobials were used. They were primarily intended to prevent necrotic enteritis caused by *Clostridium perfringens* and coccidiosis **(CSCRA, 2016)**.

The poultry industry uses antibiotics to improve meat production through increased feed conversion, growth rate promotion and disease prevention. Antibiotics can be used successfully at subtherapeutic doses in poultry production to promote growth **(Khodambashi Emami et al., 2012; Chattopadhyay, 2014)** and protect the health of birds by modifying the immune status of broiler chickens **(Lee et al., 2012)**. This is mainly due to the control of gastrointestinal infections and microbiota modification in the intestine **(Dibner and Richards, 2005; Torok et al., 2011; Singh et al., 2013)**. The mechanism remains unclear, but antibiotics are likely to act by remodeling microbial diversity and relative abundance in the intestine to provide an optimal microbiota for growth **(Dibner and Richards, 2005)**.

For example, meta-genome sequencing approaches have demonstrated that diets with salinomycin (60 ppm) have an impact on microbiome dynamics in chicken ceca **(Fung et al., 2013)**. Similarly, the use of virginiamycin (100 ppm) as a growth promoter has been associated with an increased abundance of Lactobacillus species in broiler duodenal loop at proximal ileum. This indicates that virginiamycin alters the composition of chicken gut microbiota **(Dumonceaux et al., 2006)**. In addition, populations of Lactobacillus spp. in the ileum of chickens receiving feed containing tylosin, a bacteriostatic, are significantly lower than those in chickens receiving no tylosin **(Lin et al., 2013)**. For reminder, Lactobacillus are the primary commensal bacteria to produce bile hydrolase salt. The decrease in the Lactobacillus population in antibiotic-treated animals probably reduces the intestinal activity of the bile hydrolase salts, which would increase the relative abundance of conjugated bile salts, thus promotes lipid metabolism and energy harvesting and increases animal weight gain **(Lin et al., 2013)**.

In addition to bio-resistance, antibiotics abuse has resulted in drug residues in animal products **(Gonzalez Ronquillo and Angeles Hernandez, 2017)**. Several antibiotics such as penicillin, tetracycline, macrolide, aminoglycoside and amphenicol have been detected in foods **(Diarra and Malouin, 2014)**. Residues in livestock production can have adverse impact on human health; this is the case for tetracyclines, which interfere with teeth development in young children **(Kümmerer, 2009)**. This is also the case with beta-agonists, such as clenbuterol, leading sometimes to food poisoning and muscle tremors, palpitations, and tachycardia **(Chan, 1999)**. Clenbuterol is prohibited in EU. Some breeders use it to produce meat containing less fat and more protein. Further, chloramphenicol illustrates both potential problems **(Gassner and Wuethrich, 1994)**. **Gassner and Wuethrich (1994)** have demonstrated the presence of chloramphenicol metabolites in meat products. These authors concluded a possibility link between the presence of these antibiotic residues in meat and the occurrence of aplastic anemia in humans.

The administration and restriction of the use of antibiotics in the EU are regulated by Directive 96/23/EC **(European-Commission, 1996)**. This directive focuses on measures to monitor residues in animal products. Certain limits of antibiotic residues are imposed in food and animal products (meat and eggs).

The global consumption of antibiotics in human and animal production is estimated between 1×10^5 and 2×10^5 t (**Manzetti and Ghisi, 2014)**. Releasing thereby large quantities of antibiotics into the environment entertains the cycle of biotransformation and bioaccumulation of antibiotics in the environment. According to **Manzetti and Ghisi (2014)**, the most vulnerable ecosystems to antibiotic contamination are confined aquatic ecosystems such as ponds and lakes and soils close to urban sites. Aquatic compartments, such as water and sediments, can thus play an important role in the transfer, evolution, and ecology of antibiotic resistance genes **(Marti, Variatza and Balcazar, 2014)**. Large amounts of antibiotics administered to animals are excreted into the environment via urine and faeces. After metabolic changes in animals, 30 to up 90% of the dose consumed is found in the urine and feces as parent compounds and/or metabolite compounds **(Carvalho and Santos, 2016)**. This makes sewage disposal systems one of the most important routes by which antibiotics can enter in the environment **(Gonzalez Ronquillo and Angeles Hernandez, 2017)** and contaminate even coastal waters **(Chen et al., 2015)**.

Antibiotics' risks in the aquatic environment and sediments are important because they can influence aquatic life behavior. Antimicrobials have qualitative and quantitative effects on the microbial community residing in sediments, which in turn can affect the degradation of organic matter **(Kümmerer, 2009)**. Residues of antibiotics present in the water contribute strongly to the maintenance, emergence, and dissemination of bacterial populations with a low level of resistance and are ready to evolve towards resistance **(Corvaglia, 2006)**. According to **Chen et al. (2015)**, the spatial distribution of antibiotics in the marine environment is significantly correlated with environmental variables such as chemical oxygen demand and nitrates.

The aquatic environment is considered as an important point for acquisition and spread of antibiotic resistance genes by bacteria **(Devarajan et al., 2017)**. Studies **(Caplin et al., 2008; Devarajan et al., 2017)** demonstrated a widespread presence of antibiotic resistance determinants in aquatic sediment ecosystems. They also reported multi-resistance profiles in Pseudomonas spp. in aquatic sediment samples, which is potentially transferable to humans. **Laroche et al. (2009)** reported resistance genes in estuary samples, mainly carried by *Enterococcus* spp. and *E. coli*. In addition, a study **Furtula et al. (2013)** carried out on samples collected from 2 poultry farms showed that 58% of *Enterococcus* spp. isolates in surface waters and 100% of isolates in groundwater were resistant to more than an antibiotic. According to **Carvalho and Santos (2016)**, the toxic effects of antibiotics in the aquatic environment increased when combined with other antibiotics.

In the soil, antibiotic's behavior differs according to their physicochemical properties, soil characteristics, as well as climate conditions. Acid rain accelerates the antibiotics accumulation in animal manure and soil surface while long-lasting rains foster antibiotics' migration in deeper parts of the soil **(Pan and Chu, 2017)**. According to them, antibiotics leaching is higher in sandy soils than in clay and silty soils. Norfloxacin and tetracycline tend to persist in the soil surface while sulfamethazine and erythromycin pose a higher risk for deeper soil layers and groundwater. The soil can be also contaminated by antibiotics in litter. Animal bedding contains residues of antimicrobial compounds. Residues of bacitracin, salinomycin, penicillin and virginiamycin were detected in chicken litter at concentrations ranging from 0.07 to 66 mg.L^{-1} **(Furtula et al., 2010)**. When this bedding material is used as nitrogen amendment, the resistant bacteria can live in the soil for several months **(Merchant et al., 2012)**. According to **De Liguoro et al. (2003)**, biotransformation and biodegradation of antibiotics on agricultural sites can take up to 150 days. In addition, antibiotic by-products in the environment remain bioactive and can be potentially more toxic, stable, and mobile than their parent compounds **(Carvalho and Santos, 2016)**. Bio-resistant bacteria (Staphylococcus xylosus) have also been reported in air in broiler farms **(Vela et al., 2012)**. **Liu et al. (2012)** have shown that airborne transmission causes the spread of epidemic diseases and poses impend over public health.

Scientific evidence suggests that the use of antimicrobials in livestock production can promote bacterial resistance in treated animals **(O'Brien, 2002)**. Antibiotic resistance is defined as the ability of microorganisms to proliferate in presence of an antibiotic that generally inhibits or kills microorganisms of the same species. Resistance is by mutation or acquisition of genes carried by mobile genetic elements such as transposons, integrons, plasmids or phages **(Kempf and Zeitouni, 2012)**.

Chicken harbors large proportion of *Enterobacteriaceae* resistant to aminosides in its digestive tract and tetracycline in its meat **(Yulistiani et al., 2017)**. Bacterial resistance to antibiotics has been the subject of several studies in the recent years **(Forgetta et al., 2012; Furtula et al., 2010, 2013)**.

In one study on *Salmonella enterica* isolates collected from poultry farms in British Columbia (Canada), **Diarra et al. (2014)** showed that more than 43% of the isolates were simultaneously resistant to ampicillin, amoxicillin-clavulanic acid, ceftiofur, cefoxitim and ceftriaxone. Another Canadian study **(Diarra and Malouin, 2014)** highlights the existence of different stereotypes of Salmonella, isolated from broiler farms, resistant and multi-resistant to antibiotics.

In addition, antibiotic resistance in *Enterococci* **(Silbergeld et al., 2008)**, Mycoplasma gallisepticum **(Pakpinyo and Sasipreeyajan, 2007)** and Salmonella spp. **(Manning et al., 2015)** isolated in broilers have been reported. A study in Germany **(Schwaiger et al., 2012)** showed that resistant and multi-resistant isolates are very common in chicken meat. Another study in Italy **(Bacci et al., 2012)** reported that 86% of *S. enterica* isolated from chicken carcasses were resistant to tetracycline, while 30% of isolates showed multipharmacological phenotypic resistance to ampicillin, sulfamethoxazole, and tetracycline. In Ecuador, a study by **Braykov et al. (2016)** showed that tetracycline resistance was detected in 78% of production bird (broilers and laying hens). More than half of the isolates were resistant to sulfisoxazole and trimethoprim-sulfamethoxazole (69 and 63%, respectively).

Bacterial resistance to animal antibiotics is a public health issue. In Canada, for example, poultry meat may play a role in human infections **(Diarra et al., 2010; Manges et al., 2007)**. In addition, **Hur et al. (2011)** founded that isolates of S. enterica from egg and chicken carcasses were resistant to penicillins, sulfisoxazole, treptomycin, tetracycline and quinolones. *S. enterica* isolates were resistant to at least 21 antibiotics used by the authors.

The abusive use of antibiotics and the associated selection pressure have led to decreased therapeutic efficacy and created populations of antibiotic-resistant microorganisms. Antibiotic resistance may spread over time despite the suspension of antibiotic use. Indeed, strains of E. coli resistant to trimethoprim and streptomycin have been shown to persist for several weeks in a chicken farm without using the antibiotics mentioned above **(Chaslus-Dancla et al., 1987)**.

On the other hand, antibiotic resistance is lower in organic farms (Hegde et al., 2016). Thus, it is imperative to determine the exact sources and ecology of resistant bacteria to develop strategies to stop their proliferation **(Diarra and Malouin, 2014)**.

1.4.4 Plant residues in animal feed

Consumers' pressure and worries towards harmful effects of antibiotic use and the ban of antibiotics in EU have prompted researchers to think about alternatives to antibiotics (Diarra and Malouin, 2014). The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. There are indeed several non-therapeutic alternatives that can substitute antibiotics use. Among these, the most popular are probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocins, bacteriophages, phytogenic feed additives, phytocides, nanoparticles essential oils and residues of plant processing **(Mehdi et al., 2018)**.

Due to population growth, more food production is required to improve food security **(Hartikainen et al., 2018)**, so those involved in the nutrition of animals intended for human consumption are challenged to optimize food efficiency, obtaining more meat, milk, and egg but with less feed for the animals' diet **(Makkar, 2016)**. There is also a need to restrain and reduce the environmental impact of livestock, using less land, water, energy, and fertilizers

(Röös et al., 2017) and reducing greenhouse gas emissions **(Salami et al., 2019)**. A strategy to achieve these objectives is to use the waste generated by agriculture, such as plant residues or by-products of the food industry **(Ulloa et al., 2004)** to feed animals intended for human consumption **(Westendorf et al., 1996)**. Derived from the demand for food of animal origin for human consumption, production systems require a shift toward more sustainable livestock, based on the efficient use of available food resources, reducing waste, and using new food sources, particularly those that are not destined for human consumption **(Wadhwa and Bakshi, 2013)**. Under this context, plant residues is key to achieve this objective. In fact, the use of plant waste for animal feed is not new, as it is a common practice in rural areas **(Angulo et al., 2012)**.

The incorporation of plant waste into animal feed could reduce production costs **(Wadhwa and Bakshi, 2013)**. This is particularly important as feeding represents one of the most expensive items of livestock systems. However, it is necessary to emphasize that the use of plant residues in animal feeding does not always imply a reduction in production costs since factors such as the processing itself can increase the price of the diet. For example, due to their high moisture content, agricultural residues require drying to be preserved for long periods of time. The environmental benefit must be considered by decreasing the amount of waste that reaches the landfills **(Cook et al., 2018)** and a lower proliferation of insects, which find plant waste an ideal place for reproduction **(Beausang, Hall and Toma, 2017)**.

Currently, there is no estimate on the amount of agricultural waste that is generated, mainly because of the absence of records and the fact that the harvest seasons are different every year **(Röös et al., 2017)** and that waste has no economic value since it does not have a place in any market. However, the FAO notes that one-third of all food produced is wasted **(Beausang, Hall and Toma, 2017)**, being the highest waste rate from fruit and vegetables **(Gustavsson et al., 2012)**, and more than 40% of the losses of foods are generated in the post-harvest and processing stages **(Girotto, Alibardi and Cossu, 2015)**. China is one of the countries that generate more plant waste, producing about 32 million tons annually, while the United States of America produces 15 million tons, which at best, are used in compost production or simply are deposited in landfills or dumpsites **(Wadhwa and Bakshi, 2013)**. The use of plant waste as soil fertilizer, as raw material to generate biofuels **(Beausang, Hall and Toma, 2017)**, and as feed for small farms has also been reported **(Hartikainen et al., 2018)**. In this way, plant waste can continue to be part of the human food chain **(Beausang, Hall and Toma, 2017)** as livestock could recycle nutrients from this type of waste, which are no longer suitable for human consumption, by converting them in nutritious foods. Thus, using plant waste as animal feed avoids competition between foods and fodder **(Salami et al., 2019)** as well as contributing to the efficient use of local agricultural resources **(Röös et al., 2017)**.

A major limitation of plant waste is its high moisture content that makes handling difficult and favors its rapid decomposition. Therefore, it is necessary to use some form of conservation of this waste **(Ulloa et al., 2004)**, without diminishing its quality **(Esteban et al., 2007)**. In addition, it should be considered that plant residues are not always produced constantly throughout the year, thus most of the production is available only during certain months of the year **(Acosta-Martínez, Avendaño-Ruiz and Astorga-Ceja, 2015)**. Consequently, plant residues could be a feed alternative during the dry season when other resources are depleted **(Valbuena et al., 2015)**. Silages are an option that could be used to conserve plant residues for a longer time **(Hossain et al., 2015; Chavira, 2016)** as there are examples of their application in various fruits and vegetables, such as cassava, beets, carrots, broccoli, squash, various citrus fruits, banana, and pineapple **(Diaz Monroy et al., 2014; Zivkov-Balos, Jakšić and Jovicin, 2015; Caicedo et al., 2017)**. However, due to its high moisture content, plant residues cannot be silage alone, so they are mixed with straw or stubble **(Wadhwa and Bakshi, 2013)**.

Plant residues contain various bioactive compounds, such as vitamins, unsaturated fatty acids, and phytochemicals, which can benefit the health and productivity of animals **(Salami et al., 2019)**. Several assessments have shown that plant residues can be used as part of the livestock diet, replacing part of other ingredients, without affecting weight gain, milk production, or egg-laying **(Angulo et al., 2012; Bakshi, Wadhwa and Makkar, 2016)**. In general, the peel, pulp, and seeds of vegetables are a source of polyphenols, which have anticancer, antimicrobial, antioxidant, and immune system stimulant properties. Essential oils are obtained from the peel of some citrus fruits that can prolong the shelf life of food. Plant residues can also contain antioxidants that act by eliminating free radicals and preventing the formation of peroxides. Additionally, plant residues can be a source of various enzymes such as bromelain (pineapple), papain, amylase, laccase, and manganese peroxidase, which have various biological functions and biotechnological applications **(Wadhwa and Bakshi, 2013)**.

1.4 Meat performance of broiler chickens

Poultry meat mainly comes from standard production system using high growth rate strains reared under indoor intensive conditions. However, it is possible to find also different alternative systems using outdoor extensive rearing conditions and slow-growing lines. These different production systems can affect carcass and meat quality **(Baéza, Guillier and Petracci, 2021)**.

The genetic selection for breast meat yield, for example, has been effective in producing carcasses with higher meat yield, but resulting since a decade in the increased occurrence of quality defects and myopathies (white striping, wooden breast, spaghetti meat and deep pectoral disease). Outdoor access has positive effects on the image and nutritional properties (through its effect on the fatty acid profile of meat lipids), but it increases the exposition risk to environmental contaminants and pathologies (parasites, virus, bacteria); it also increases the variability in meat quality linked to the variability of animal performance and slaughter age. The orientation towards more agro-ecological low-input farming systems may present benefits for the image and nutritional properties, but also risks for the commercial (low carcass weight and low breast yield, irregularity in supply), organoleptic (stronger flavour, less tender and darker colour of the meat) and in terms of variability of the different properties that constitute quality **(Baéza, Guillier and Petracci, 2021)**.

The poultry market standard items depend on retailer and consumer requirements such as slaughter weight, RTC carcass yield, and carcass appearance (absence of visual defects such as inconsistent skin pigmentation, scratches, skin lesions, blisters, bruises, fractures, cellulitis, etc.). The slaughter weight and RTC carcass yield depend on strain, sex, farming and management system, and slaughter age while carcass appearance depends on diet, and rearing, preslaughter and slaughtering conditions. In a study carried out in 2012–2013 among five slaughterhouses throughout France, the rate of carcass downgrading for "outdoor" and conventional chickens was 0.49% and 1.36%, respectively **(Baéza et al., 2015a)**. In a survey carried out on the technico-economic results obtained in 2017 in the west part of France, the rates of carcass downgrading were higher for export chickens (2.21%), conventional (0.96%) and heavy (0.88%) chickens compared to certified (0.40%), "Label Rouge" unconventionally reared (0.36%) and organic (0.42%) chickens **(Chambres d'Agriculture de Bretagne, 2019)**.

The poultry breast yield is also an important criterion considered by breeders and processors due to its economic valuation. Currently, the fillet yield of slow-growing chickens is around 16% and that of fast-growing strains is around 22%. The extraordinary breast yield increase obtained in modern hybrids was mainly achieved through selection **(Petracci, Soglia and Berri, 2017)**.

According to **Haščík et al. (2009)**, the technological quality of poultry meat is assessed according to external characteristics (quantitative assessment) and according to qualitative characteristics. External features include the weight of the slaughtered poultry, the meatiness, the balance of the various body parts, but also the balance between the sexes and the individuals, while the skin color, the skin surface and the carcass processing are also evaluated. The basic carcass úarts of poultry are the breasts, thighs, back and wings. When determining the carcass value, we determine the carcass yield, edible proportion, respectively individual proportions of valuable meat parts from the whole carcass.

Meat quality is a complex peculiarity that is influenced by genetic and environmental factors. Changes in meat quality and between individuals can be large (Rehfeldt et al., 2004).

There are many aspects that affect the overall quality of poultry meat, such as genotype, age, food, production system, temperature changes, photoperiod, activity level and others **(Fanatico et al., 2005)**.

Čuboň, Haščík and Kačániová (2009), **Haščík et al. (2010)** and other authors have addressed in the past the issue of the dynamics of growth in live body weight of various hybrid combinations broiler chickens, as well as the structure of the carcass, respectively proportion of the most valuable meat parts, i.e. breast and thighs from carcass, which may be affected by age, genetics, feeding, husbandry conditions and other internal and external factors.

Proportion of valuable meat parts in chickens is expressed in percentages, on average the breasts are represented by 28.7%, thighs by 32.1%, back by 27.6% and wings by 11.6%. These indicators are influenced by environmental conditions, such as fattening duration, nutrition, housing conditions, microclimate, health status and others **(Brázdová, 1996)**.

According to **Kaněra (1995)**, between 1984 and 1994, the live body weight of Ross broilers at 42 days of age was increased by an average of 70 g per year and the amount of beast muscle increased by 0.2%. The author states that the hybrid combination Ross 208 chickens has better production abilities, but Ross 308 chickens have a higher proportion of breast muscle from the total carcass weight, so it is especially suitable for markets where the purchase price is governed by the amount of pectoral muscle produced. In our conditions, the author recommends Ross 208 hybrid combination for more efficient fattening.

The chickens of the Ross 208 hybrid combination reached a live weight of 1769.5 g in 13 replicates, the proportion of breast muscle from the live weight was 19% and the carcass yield was 74.2%. The Ross 308 hybrid combination in 6 replicates achieved a live weight of 1798.2 g at 35 days, a breast muscle content of 19.1% and a carcass yield of 73.5%. The hybrid combination AVIAN 34 in 2 replicates reached a live weight of 1838.7 g, the proportion of breast muscle from the live weight was 17.9% and the carcass yield 71.9% **(Machander and Činčurová, 2000)**.

Novel et al. (2009) found a live weight of Ross 308 hybrid at 21 days of age at 432 g in males and 415 g in females. The daily feed consumption up to 21 days of age was 65 g for males and 64 g for females. At 42 days of age, the live weight of the males was 1506 g, the females 1348 g, the breasts weight of males 268.5 g, females 252.8 g and the thighs reached 82.8 g (males) and 74.8 g (females). The feed conversion was 2.2 kg for males and 2.3 kg for females. The average daily feed consumption from hatching to day 42 of fattening was 106 g for males and 101 g for females.

Nakalebe et al. (2009) in their experiment with Ross 308 chickens reached a live weight of 42 days at 1579 g, a breast weight of 362 g and a thigh weight of 120 g.

Haščík, Kačániová and Mihok (2010) performed a feeding experiment with commercial feed compound with Ross 308 chickens and found live weight 1644.1 g and Cobb 500 chickens slightly lower at 1629.2 g at 35 day of age.

In a similar experiment, but when applying the saccharide raw material, **Liptaiová et al. (2009)** found the live weight of broiler chickens at 37 days of age in Ross 308 hybrid 1746.7 g and Cobb 500 hybrid 1611.3 g.

Based on the results of the experiment of Kumar et al. (2010) in Cobb 400 chickens, live body weight at 42 days of age was 2074 g – 2173 g.

Makram et al. (2010) found at 42 days of age live body weight of the Avian 2498.3 g, Cobb 2434.6 g, and Hubbard JV 2391.4 g hybrid combination. The carcass weights of the hybrids observed were Avian (1852.6 g), Cobb (1793.3 g) and Hubbard JV (1773.7 g) at the same feeding.

Barteczko and Lasek (2008) found values of live weight at the age of 49 days in Ross 308 chicken when applying different amounts of protein in compound feeds at levels 2108 to 2307 g.

In the experiment, **Horniaková (1998)** monitored meat performance indicators chickens of the Hybro hybrid combination for 42 days. The best results have been achieved in experimental groups with a higher proportion of mineral additive and fishmeal. The average carcass weight was 1681.8 g, the carcass yield was 77.6% and the breast muscle weight was 338.3 g. The average feed consumption per kg gain was 2.07 kg.

Influence of sex at the same age on live weight, carcass value and meat quality of Hybro broiler chickens on deep litter until age of 49 days was observed by **Horváthová et al. (1998)**. The chickens achieved an average carcass yield 72.6%. Gender has affected carcass yield – females reached normative carcass yield in each weight group and males at live weights above 1700 g. The proportion of breast and thigh muscles was also significantly affected, while the proportion of thighs was higher in females (77%) and the proportion of bones was significantly higher in males (21.6%). The proportion of the consumable share was demonstrably higher in females (79.7%). Females stored more subcutaneous fat (18.6%) than males (17.1%), so the energy value of chicken meat was higher in females (1041.5 kJ). The share of the back and wings represented on average 38.5% of the fuselage weight. The authors found that the live weight mainly affects the proportion of subcutaneous fat, while its proportion increases with increasing live weight and decreases the proportion of whole proteins, but at the same time with increasing live weight, carcass yield significantly increases.

Feeding trials with rapeseed meal, fermented with *Lactobacillus fermentum* and *Bacillus subtilis*, showed improved weight gain and feed conversion ratio (FCR) in broiler chickens **(Chiang et al., 2010)**. However, in another study, no health influences on broiler performance were observed by **Xu et al. (2012)** when soybean was replaced by up to 10% rapeseed meal fermented with *L. fermentum* and *B. subtilis*. Fermentation of other feedstuffs, e.g., *Aspergillus oryzae* fermented soybean meal **(Mathivanan, Selvaraj and Nanjappan, 2006; Feng et al., 2007)** and lactobacilli-fermented wheat and barley **(Skrede et al., 2003)** resulted in improved broiler performances when compared to broilers fed on nonfermented control diets. As demonstrated by **Skrede et al. (2003)**, weight gain of broilers was higher by 182 to 232 g when fed fermented barley, and higher by 50 to 108 g when fed fermented wheat than that of broilers fed corresponding control diets. The authors also reported 29% lower soluble b-glucans after barley fermentation, but this was increased by 12% for wheat. In line with this observation, **Skrede et al. (2003)** suggested that the degradation of b-glucans in barley during fermentation was mainly associated with greater influences of fermented barley on body weight, when compared to fermented wheat. Mentioned results suggests that improvement in nutritive values and the digestibility of feed ingredients through soluble NSP degradation during fermentation is a major factor underpinning improved broiler performances.

Other than growth performances, breast meat yields and abdominal fat deposition are other concerns when feeding unconventional diets to broiler chickens, as these parameters are often used as key measures of meat production **(Widjastuti, Aisyah and Noviadi, 2010)**. In the study by **Kayode et al. (2012)**, the inclusion of *Aspergillus niger* and *Penicillium chrysogenum*-fermented mango kernel cake, in up to 60% of the diet, had no impact on breast meat yields and abdominal fat deposition, when compared to controls. Similarly, the authors also showed that inclusion of fermented mango kernel cake, in up to 20% of the diet, did not impact carcass weight.

A similar result was observed by **Santoso et al. (2004)**, where the inclusion of Lactobacillus spp.-fermented (layer) faeces, in up to 15% of the diet, did not influence carcass weights and abdominal fat deposition in broiler chickens. Moreover, **Widjastuti, Aisyah and Noviadi (2010)** reported that inclusion of A. niger-fermented waste-cassava leaf meal, of up to 25% of the diet, had no influence on carcass weights and carcass percentages. **Mathivanan, Selvaraj and Nanjappan (2006)** also reported that inclusion of A. niger-fermented soybean meal, of up to 1.5% of the diet, had no impact on the ready-to-cook weight or the live weight of broilers.

Unlike these studies, lower breast meat yields (percentage of live body weight) were recorded in birds fed fermented moist feeds in the study by **Missotten et al. (2013)**. In their study, although treatments did not affect the dressing percentage of broilers, **Skrede et al. (2003)** found that abdominal fat increased with increasing levels of Lactobacillus strain AD2-fermented barley in administered feeds. Likewise, **Zhang et al. (2016)** reported that the inclusion of 6% fermented feed into the diet resulted in increased abdominal fat percentages of 56-day-old broiler chickens. In contrast to these last two studies, **Nie et al. (2015)** reported that feeding cottonseed meal fermented by *Candida tropicalis* and *S. cerevisiae*, decreased abdominal fat relative weight (due to increased fatty acid b-oxidation and triglyceride hydrolysis) in broilers.

1.5 Nutritional quality of chicken meat

The quality of meat is built and can deteriorate along the continuum from the conception of the animal to the fork. Different factors implicate in the determinism of poultry meat properties and pinpoint critical periods, such as the preslaughter and slaughter periods, and key factors, such as the feeding regimen, via its direct effect on the fatty acid profile, the antioxidant and volatile compound contents, and indirect effects mediated via the growth rate ofthe bird **(Baéza, Guillier and Petracci, 2021)**.

Poultry meat and poultry products are of great importance in human nutrition worldwide, as their availability contributes to addressing global food shortages. They provide an excellent source of proteins, fats, essential amino acids, minerals, vitamins, and other nutrients **(Shaltout, 2019)**. The indistinct taste and high tenderness of chicken breasts enable the production of a variety of meat products, which are aimed at different groups of consumers. In addition, chicken breast meat is suitable for quick and undemanding preparation, which is especially popular nowadays, when people tend to spend less and less time preparing meals at home **(Petracci et al., 2013)**.

The great advantage of chicken meat is that, unlike other types of meat, there are no religious and social restrictions for its consumption **(Haščík et al., 2020)**. In addition, it is relatively cheap. It is generally more consumed by the inhabitants of cities than in the countryside, which are dominated by cereals. However, most cereals are poor in full-fledged proteins, resp. essential amino acids such as lysine, threonine and sulfur methionine and cysteine that are present in chicken meat. Compared to the red meat of large farm animals, chicken is generally considered healthier. The breast muscle of chickens contains less than 3 g of fat per 100 g of meat, while red meat contains approximately 5 to 7 g of fat per 100 g. About half of these fats are valuable monounsaturated fatty acids and only one third are less healthy saturated fatty acids. In red meat, the most saturated fat is the most saturated fatty acids **(Dowarah, 2013)**.

1.5.1 Proteins and amino acids

Chicken meat has high protein content around 23 – 25% in the fillet and 18% in the thigh **(Berri et al., 2005, Baéza et al., 2012)**. The main amino acids are glutamine, asparagine, lysine, leucine, arginine, and alanine. The amino acid composition of poultry meat is relatively stable. However, it can vary slightly by modulating the amino acid intake of the diet. Increasing the dietary level in valine, isoleucine, and leucine to 150% of the growth requirement, 10 days before slaughter, increased the glutamate content (precursor of the umami aroma sought in some Asian countries) of chicken fillets (+30%) compared to fillets of chickens fed a diet at 100% of the growth requirement.

The protein content of poultry meat is mainly influenced by the slaughter age. For a heavy strain of conventional chickens, the protein content of fillets increased from 23.5% to 24.9% between 35 and 63 days of age **(Baéza et al., 2012)**. The protein content of mule duck fillet increased from 20.6 to 22.4% when animals were slaughtered at 8 or 13 weeks of age **(Baéza et al., 2000)**. With selection on the growth rate, the slaughter age of conventional chicken is steadily decreasing. This results in an increased ratio of moisture/protein contents of the fillets, which according to European legislation should not exceed 3.40. It was showed that, in Germany, for conventional chicken production, this ratio increased from 3.10 in 1993 to 3.31 in 2012, with an increased proportion of fillets exceeding the legal limit value. Age will also affect the collagen content of the meat. This content is halved in the Muscovy duck fillet between 8 and 12 weeks of age **(Baéza et al., 2002)**. In chicken fillets and thighs, it varies between 8.7 and 7.3 $mg.g^{-1}$ and 16.1 and 17.4 $mg.g^{-1}$, respectively, between 8 and 16 weeks of age **(Touraille et al., 1981)**. Recently, with the appearance of growth-related abnormalities such as “white striping” or “wooden breast”, there has been a significant decrease in muscle protein content and an increase in collagen content associated with their occurrence **(Mudalal et al., 2014, Mazzoni et al., 2015)**.

1.5.2 Lipids and fatty acids

The most variable fraction concerns lipids. The average fat content is 1.3% in the fillet and 4.5% in the thigh of conventional chicken **(Rabot, 1998)**. Turkey and “Label Rouge” unconventionally reared chickens’ meat is leaner (0.8% in the fillet). Duck meat is fatter (1.5 – 2% in the fillet depending on the species; **Baéza, 2000**). Within lipids, the most variable fraction is that of triglycerides, the amount of which is positively correlated with that of the lipid content: 0.7% in the fillet and 3% in the thigh of chicken **(Rabot, 1998)**, 0.5 – 0.8% in the duck fillet **(Baéza, 2000)**. Phospholipid content is 0.6% in the fillet and 0.8% in the thigh of chicken **(Rabot, 1998)**. In duck fillet, this content is 1.1% **(Baéza, 2000)**. The cholesterol content is 0.05% in the fillet and 0.09% in the thigh of chicken (Rabot, 1998). It is comprised between 0.07 and 0.12% in the duck fillet **(Baéza, 2000)**.

Feed will affect the intramuscular lipid content mainly by the energy content of diets and especially by the energy/protein ratio or when the intake of essential amino acids (lysine, methionine) is lower than the requirement for growth. By increasing feed lipid content by 30 or 90 $g.kg^{-1}$, the lipid content of male turkey fillet was increased by 14% and 40% and that of thigh by 27% and 53% compared to the control, in parallel with an increase in BW **(Salmon and Stevens, 1989)**. In this same study, when the energy/protein ratio increased from 65 to 83 KJ/g, the lipid content of the fillet decreased by 27% and that of the thigh by 5%, in parallel with a decrease in BW. **Conde-Aguilera et al. (2013)** compared two levels of dietary methionine intake in chickens between 7 and 42 days. By reducing this intake by 34% compared to growth requirement, the fillet lipid content was increased by 28% at the age of 42 days. In contrast, the energy source of the diet (carbohydrates or fats) had no effect on the intramuscular fat content of chicken **(Baéza et al., 2015b)**. The dietary restriction decreases intramuscular lipid content, while overfeeding, practised only in ducks and geese, will double this content **(Baéza, 2000)**.

The lipid content of poultry meat is also influenced by age, genotype, and production system. For a heavy strain of conventional chicken, the fillet lipid content increased from 1.29% to 1.68% between 35 and 63 days of age **(Baéza et al., 2012)**. In male mule duck, the fillet lipid content increased from 1.79 to 2.74% between 8 and 13 weeks of age **(Baéza et al., 2000)**. Comparing five genotypes of chickens with different growth rates, and therefore different slaughter ages and slaughter weight of 1.5–2 kg, **Tang et al. (2009)** showed that the average lipid content of breast and thigh muscles ranged from 0.96 to 1.42%. Old breeds of chickens with very slow growth rates are generally fatter than commercial hybrids because they have not been selected against fattening. This is the case, for example, of Géline de Touraine chicken, which had lipid content in the fillet and thigh of 1.2% and 10.5%, respectively at 84 days of age, whereas for a strain used in unconventionally reared chickens production and reared under the same conditions, these contents were 0.9% and 7.0%, respectively **(Baéza et al., 2010)**. During the last decade, there was observed an increase in meat lipid content of breast meat affected by growth-related abnormalities **(Mudalal et al., 2014, Mazzoni et al., 2015)**. A survey, carried out in several French slaughterhouses, showed that the fillet lipid content was 0.89, 1.07 and 1.31% for “Label Rouge”, certified and conventional productions, respectively. Slow-growing chickens were reared in a closed building or with free-range access. The thigh lipid content was higher for chickens reared in confinement (10.3 vs. 8.5%) while the fillet lipid content (2.1 vs. 1.9%) was not affected by the rearing system.

Fatty acids (FAs) in chicken meat consist of approximately one-third of saturated fatty acids (SFAs), one-third of monounsaturated fatty acids (MUFAs) and one-third of polyunsaturated fatty acids (PUFAs) (Rabot, 1998). Oleic acid is the main MUFA (5/6) followed by palmitoleic acid (1/6). The main PUFAs are linoleic and arachidonic acids. Total lipids in chicken muscles also contain linolenic acid and long-chain PUFA from *n*-6 and *n*-3 series. The main factor of variation in poultry meat FA composition is the feed FA composition. Palm and copra oils increase the proportions of short-chain and saturated FAs; animal fat enriches the lipid deposits of chicken with palmitic and stearic acids. Conversely, with vegetable oils, the proportions of PUFAs with 18 carbon atoms increase. On the other hand, marine oils significantly increase the proportions of long-chain *n*-3 PUFAs **(Lessire, 2001)**.

Since (2001), the replacement of animal origin fats (tallow and lard) by vegetable oils (rapeseed, soya, or flax) in poultry feed increased the proportion of PUFAs in poultry meat. Now, conventional chickens have high meat content in PUFAs (30.02% in the fillet), due to a higher dietary and also have high lipid content in meat (1.25%) **(Chartrin et al., 2005)**.

In Western countries, the daily intake in FAs is not satisfactory because the ratio of *n*-6 FAs/*n*-3 FAs is around 15, while a value of five is recommended. Several studies have been undertaken to enrich fresh chicken meat with *n*-3 FAs. The use of fish oils rich in long-chain *n*-3 PUFAs is the most effective way. Fish oils can be replaced by microalgae. It is also possible to use linseed or rapeseed oils that are rich in linolenic acid, although in this case, the proportion of long-chain *n*-3 PUFAs deposited in the muscles remains low. **Baéza et al. (2015a)** showed that the combination of extruded flaxseed with microalgae in feed for conventional chicken succeed to enrich the meat with linolenic acid and long-chain *n*-3 FAs with a *n*-6 FA/*n*-3 FA ratio of 3.65 in comparison with a soy oil-based diet, rich in *n*-6 FAs and a *n*-6 FA/*n*-3 FA ratio of 11.52.

A previous study showed that feeding fermented feeds appeared to improve the fatty acid profiles of chicken meat. **Marcinčák et al. (2018)** reported that feeding 10% cornmeal, fermented with *Umbelopsis isabellina* CCF2412, resulted in increased proportions of gamma-linolenic, alpha-linolenic, and oleic acids in breast meat fat, and improved ratios of n-6 to n-3 polyunsaturated fatty acids in the raw meat. These authors also documented that fermented feed improved the quality, oxidative stability, and sensory properties of broiler meat. Unlike this study, **Chung and Choi (2016)** reported that feeding 1% fermented red ginseng marc with red koji, did not affect fatty acid profiles in breast and thigh muscles of broiler chicks. It appears that the different nature and levels of fermented feeds, and the performance of in vivo trials may explain these divergent results.

Numerous studies have also investigated the enrichment of poultry meat with conjugated linoleic acids (CLAs). **Du and Ahn, (2002), Sirri et al., (2003)** tested different levels of dietary CLA intakes in chicken from 0.25 to 4% for 3 to 5 weeks. The deposition of CLA in meat increased with the increasing feed CLA content while the oleic, palmitoleic and arachidonic acid contents decreased. A combined dietary intake of CLA with fish oil increased the efficiency of deposition in the long-chain *n*-3 PUFAs and CLA in chicken muscles compared to a single intake of CLA or fish oil.

More recently, the use of fat from insect larvae was used as an alternative fat source to soybean oil. Consequently, in the chicken meat (breast and thigh), the proportion of SFAs (particularly lauric and myristic acids) was increased to the detriment of PUFAs. The *n*-6 FA/*n*-3 FA ratio was also increased **(Schiavone et al., 2017, Cullere et al., 2019)**.

The feed energy source may also influence the FA composition of poultry meat. A high-carbohydrate diet will promote liver lipogenesis and therefore the synthesis of SFAs and MUFAs, while a high-fat diet will rather promote the direct deposit of dietary FAs in peripheral tissues **(Baéza et al., 2015b)**. In the extreme case of overfeeding, the daily intake of carbohydrates (corn starch) is very high. Hepatic lipogenesis is strongly stimulated and particularly the synthesis of palmitoleic and oleic acids that are then deposited in peripheral tissues (adipose and muscle tissues). In the fillet of overfed ducks, MUFAs and PUFAs proportions are 50 and 16% of total FAs, respectively vs. 36 and 31% in the fillet of mean ducks **(Girard et al., 1993)**.

Depending on the studies, the FA composition of poultry meat may also vary or not during storage at +4 °C or -20 °C. Cooking may have or not an effect on the FA composition. For example, **Baéza et al. (2013)** showed that curing-cooking process had few impacts on the FA composition of chicken fillets. The same was true after 30 min of cooking at 80 °C a mixture of ground turkey muscles (25% fillets, 25% thighs, 50% mechanically separated meat; **Ahn et al., 1993**). The CLA content of chicken thighs is little modified by roasting. On the other hand, it is decreased when the meat is boiled or fried **(Franczyk-Zarow et al., 2017)**. After roasting whole chicken carcasses, the composition of the meat is modified because the fatty acids of the subcutaneous adipose tissue will migrate to the meat. These are mainly SFAs and MUFAs from triglycerides **(Rabot, 1998)**.

1.5.3 Minerals and trace elements

The mineral content (mainly calcium, phosphorus and potassium) of poultry meat is 1.1% **(Rabot, 1998)**. This parameter is little affected by feeding and other rearing factors if the dietary intakes cover the animal requirements.

Several studies have been undertaken to enrich poultry meat with vitamin E but also vitamins A and C. The deposition efficiency of dietary vitamin E in muscles depends on species. It is twice as high in chicken compared to turkey **(Gong et al., 2010)**. Free-range access increases vitamin E content in chicken meat **(Michalczuk et al., 2017)**. Poultry feed is often supplemented with carotenoid pigments to increase the yellowness of chicken skin. For example, fillets and thighs of chickens fed a diet containing long-chain PUFA *n*-3 rich microalgae had a carotenoid content twice as high as those of control chickens **(Kalogeropoulos et al., 2010)**. The deposition efficiency of dietary intake carotenoid pigments depends on the genotype. The effect of a mutation on the promoter of the BCMO1 gene on the muscle's ability to store these pigments was discussed earlier **(Le Bihan-Duval et al., 2011)**. The enrichment of meat with various trace elements such as selenium and magnesium has also been tested. By distributing supplemented feed with four increasing levels of selenium (0, 0.2, 0.4 and 0.6 $mg.kg^{-1}$) to Pekin ducks from 0 to 49 days of age, **Baltić et al. (2015)** showed that the selenium content increased from 0.05 to 0.87 $mg.kg^{-1}$ in the fillet and from 0.04 to 0.64 $mg.kg^{-1}$ in the thigh. Selenium can be provided under different forms but the organic forms (selenium yeast, seleno-methionine) allow the most effective deposition in muscles compared to mineral forms (sodium selenite) (**Briens et al., 2013** in chicken). Dietary zinc supplementation does not necessarily increase the zinc content of poultry meat **(Bou et al., 2005)**.

1.6 Organoleptic quality of chicken meat

1.6.1 Meat colour

The colour of poultry meat can be affected by feed, especially when naturally occurring or supplemented carotenoid pigments are present in the feed because they are also accumulated in intramuscular fat. In a survey of several French slaughterhouses, **Gigaud et al. (2011)** showed that L*, a* and b* values were 48.07, 0.88 and 15.15 for yellow-skinned chickens vs. 49.56, −0.01 and 10.03 for white-skinned chickens. For fatty liver production, ducks are overfed exclusively with corn. Their fillets are richer in carotenoid pigments and the lipid content is doubled in comparison with lean duck fillets **(Chartrin et al., 2006)**. Its colour is rather chocolate brown while the colour of lean duck fillet is dark red. L* and b* values are 42.30 and 14.52 for fillets of overfed ducks vs. 32.37 and 9.37 for lean duck fillets **(Chartrin et al., 2006)**. Dietary supplementation with spirulina, an alga rich in carotenoid pigments, at 0, 40 or 80 g/kg had no effect on the lightness and redness of chicken fillet. In contrast, the yellowness increased from 3.5 to 12.3 **(Toyomizu et al., 2001)**. However, the ability to fix carotenoid pigments depends on the genotype. In fact, **Le Bihan-Duval et al. (2011)** found two

SNPs (Single Nucleotide Polymorphisms) on the promoter of the gene encoding beta-carotene 15,15′-monooxygenase (BCMO1), a key enzyme involved in the conversion of beta-carotene to retinal. The fillets of chickens carrying one allele (GG) are richer in carotenoid pigments (lutein and zeaxanthin) and have a higher yellowness than those carrying the other allele (AA). The meat colour especially of yellow-skin birds also depends on the total content of lipids. The higher the lipid content, the lighter the meat and the higher the yellowness. When the average lipid content of duck fillet increases from 2.6 to 5.6%, the lightness and yellowness increase from 34.4 to 41.8 and from 10.4 to 14.2 with correlations of 0.49 and 0.47, respectively **(Chartrin et al., 2006)**. Recently, it was also found that breast meat affected by white striping abnormality showed higher lightness and yellowness because of increased lipid content **(Petracci et al., 2017)**.

For white meats, the colour strongly depends on muscle glycogen stores at slaughter and postmortem evolution of pH which affects light scattering properties of the resulting meat. In conventional chicken fillets, **Berri et al. (2007)** showed that the correlation between pHu and glycolytic potential was −0.57 and with lightness it was −0.61. At the genetic level, the correlation between pHu and lightness of fillet is even stronger ranging from −0.65 to −0.91 depending on the strains studied **(Le Bihan-Duval et al., 2008, Chabault et al., 2012)**. A divergent experimental selection on fillet pHu of high growth rate chickens confirmed these relationships between this criterion and the colour of the meat **(Alnahhas et al., 2014)**. After five generations of selection, pHu values were 6.09 and 5.67 for high and low pHu lines (pHu + and pHu-, respectively). The pHu + fillets were darker ($L^* = 47.50$ vs. 52.50), less red ($a^* = -0.17$ vs. 0.05) and less yellow ($b^* = 9.49$ vs. 11.02). In addition, the selection of chickens based on increased growth rate and breast muscle yield may result in decreased redness and increased yellowness and lightness by dilution of haem pigments.

Fresh poultry meat colour changes during shelf life according to storage conditions and packaging solutions. For example, there was found a decrease in redness of chicken thigh muscles from 8–9 to 6–7 after 5 days of storage at 8 °C in an illuminated display case 12 h per day or after 9 days of storage at +4 °C in darkness. A decrease in redness was observed when storing duck fillets and thighs at +4 °C. Supplementation with vitamin E and selenium can help to reduce discoloration during cold refrigerated storage by preventing oxidation of haem proteins **(Baéza, Guillier and Petracci, 2021)**.

1.6.2 Meat juiciness

Tenderness and juiciness are the most important quality attributes of fresh meat and meat products. Increased water and/or fat content at the time of consumption are generally associated with increased juiciness. Cooking inducing a decrease in water binding capacity and loss of moisture or fat through drip would decrease juiciness. Therefore, meat juiciness is poorly affected by diet, except for factors that affect meat lipid content. Indeed, when the lipid content of the duck fillet increases from 2.6 to 5.6%, the cooking loss increased from 15 to 17.8% with a correlation of 0.54 and the juiciness score increased **(Chartrin et al., 2006)**. Meat juiciness is mainly influenced by age and genotype. **Culioli et al. (1990), Rabot (1998)** showed that conventional chicken meat cooked in whole roasting carcass (thighs and fillets) was juicier compared to unconventional reared chickens. For slow-growing chickens slaughtered at different ages between 8 and 16 weeks, **Touraille et al. (1981)** also reported a decrease with age in the juiciness score of fillets and thighs. The postmortem process may also influence the juiciness of chicken fillets, as the shorter the time between slaughter and deboning, the drier the meat is in the mouth.

1.6.3 Meat texture

Poultry are slaughtered at very early ages compared to red meat species therefore resulting meat is generally considered to be rather tender. The texture of poultry meat is mainly affected by age, genotype, rearing system, slaughter conditions and postmortem carcass processing techniques (electrostimulation, cooling rate, and slaughter-deboning interval) which will influence the evolution of pH and rigor mortis while feeding has negligible effect **(Baéza, Guillier and Petracci, 2021)**. For slow-growing chickens slaughtered at different ages between 8 and 16 weeks, **Touraille et al. (1981)** showed a decrease in tenderness of fillets and thighs with age. The shear force value of cooked fillets and thigh muscles of “Label Rouge” chickens was higher than that of conventional chickens (53 vs. 43 $N.cm^2$ and 95 vs. 80 $N.cm^2$, respectively) and the tenderness score was lower (5.66 vs. 7.13 points, **Culioli et al., 1990**). This difference is certainly due to the difference in slaughter age (6 vs.12 weeks) but other elements could intervene such as a significantly more acidic meat pH, a smaller diameter of muscle fibres **(Berri et al., 2005b)** or an outdoor access of unconventionally reared chickens that allows them to have greater physical exercise and greater muscle activity.

The texture of the meat will also depend on the *post mortem* evolution of the pH and the rigor mortis phase. When pH is greater than 6.0, the meat is classified as DFD for "Dark, Firm and Dry" and when pH is less than 5.7, the meat is classified as acid and it has the characteristics of PSE meat for "Pale, Soft and Exudative". However, this impact on texture only affects raw meat. In fact, after cooking, the opposite is observed. In two experimental chicken lines with an average pHu of 6.09 and 5.67, the shear force value of cooked fillets was 10.9 and 16.0 $N.cm^2$, respectively **(Alnahhas et al., 2014)**. Roasted fillets and thighs of pHu + chickens were also considered tenderer than those of pHu- chickens **(Alnahhas et al., 2015)**. Recently, it was found that broiler breast fillets affected by growth-related abnormalities exhibited abnormal texture. Indeed, if wooden breast showed a marked increase of toughness assessed by both instrumental and sensory analyses, white-striped and spaghetti meat breasts had slightly lower shear force value than normal fillets **(Petracci et al., 2017)**. The delay between slaughter and deboning has a very marked effect on the tenderness of cooked meat. It is recommended to wait at least 4 h before cutting the fillets from the keel bones to avoid a meat judged hard by the consumer. Chicken fillets cut 45 min or 2 h after slaughter have a higher postcooking firmness score than chicken fillets cut 24 h after slaughter (6.6 and 6.4, vs. 4.7) **(Zhuang and Savage, 2011**). Meat hardness decreases as a function of the slaughter-deboning interval. The negative effect on the meat texture of early carcass deboning just before or during the period of rigor mortis may, in some cases, be offset by a longer maturation phase. For example, Muscovy duck meat cut 0.5 h after slaughter and stored at +4 °C for 4 days is as tender as duck meat cut 4 days after slaughter **(Knust et al., 1997)**. The negative impact on the meat texture of a short delay between slaughter and deboning is verified regardless of the type of production, with a more marked effect on the fillets of unconventionally reared chickens whose meat is firmer than that of certified and conventional chickens **(Berri et al., 2006)**.

Gas narcosis, which deprives muscles of oxygen more quickly than electric narcosis, accelerates the entry into the rigor mortis phase, which shortens the delay between slaughter and deboning **(Joseph et al., 2013)**. It is also possible to reduce this delay without altering the meat texture by electrostimulation of the carcasses during the slaughter process. Electrical stimulation after bleeding is more effective than after plucking **(Zhuang et al., 2010)**. The delay between slaughter and deboning can then be reduced to 2 h. The meat texture is also influenced by the cooling temperature of carcasses. When this temperature is around 0 °C, the muscle undergoes a cold contraction phenomenon, especially during the first hour after slaughter not followed by relaxation and altering the subsequent tenderness of meat **(Papinaho and Fletcher, 1996)**.

1.6.4 Meat flavour

Meat flavour and taste are mainly thermally derived since uncooked meat has little or no aroma. Meat become flavoursome only after cooking process, and a series of thermally induced complex reactions that occur between the different non-volatile compounds of the lean and fatty tissues. Diet formulation will therefore have a strong influence on content of flavour components by modulating particularly the lipid content and composition and levels of antioxidants and water-soluble volatile compounds **(Baéza, Guillier and Petracci, 2021)**. Fillets of overfed ducks, which are richer in fat, have a higher flavour than fillets of lean ducks **(Chartrin et al., 2006)**. Duck meat is richer in phospholipids that are precursors of aromas after cooking, and it has then a flavour considered more pronounced than that of chicken meat. The same applies to thighs compared to fillets **(Rabot, 1998)**. Fatty acid composition is also important, the long-chain n-3 PUFA content. The use of fish oils at concentrations greater than 1.5% in the feed has a negative impact on the flavour of chicken meat. Feed supplementation with 2% microalgae is detected during sensory analysis of roasted chicken thighs, with an abnormal flavour qualified as fish taste **(Baéza et al., 2015c)**. On the other hand, the flavour of the fillets, which are less rich in fat, was not modified. **Bou et al. (2001)** analysed the effect of dietary lipid source on the flavour of cooked chicken thighs after 13 months of storage at -20 °C. The rancid flavour was much more pronounced for PUFA-rich linseed oil than for beef tallow or sunflower oil. Feed supplementation with 225 mg of vitamin E.kg greatly reduced this defect. **Sheldon et al. (1997)** also showed that feed supplementation with high doses of vitamin E (250–300 ppm) had a positive effect on the flavour of turkey fillets cooked after a storage for 8 days at +4 °C or 90 days at -20 °C. It also reduced the formation of unpleasant flavours after cooking chicken thighs previously stored for 3 or 5 days at +4 °C or 5 or 10 weeks at -20 °C **(O'Neill et al., 1998)**.

The meat flavour and taste are also influenced by age, sex and genotype by their effect on lipid content and composition. **Touraille et al. (1981)** showed that the flavour of chicken fillets and thighs increased between the ages of 8 and 14 weeks. Comparing slow- and fast-growing chickens slaughtered at 48 or 83 days, **Farmer et al. (1997)** also observed an increase in fillet flavour with age. However, **Culioli et al. (1990), Girard et al. (1993), Rabot (1998)** did not reveal any difference in flavour of unconventionally reared roasted chickens fillets and thighs compared to those of conventional chickens. The selection on chicken fillet pH also had an impact on the perception of the acidity of roasted or grilled fillets, the chickens of the experimental pH - line having a higher score than pH + chickens **(Alnahhas et al., 2015)**. Finally, the storage duration and conditions may also have an impact on the meat flavour. For example, **Haugen et al. (2006)** noted the development of the rancid odour of skin-free turkey thigh muscles, crushed and stored at -10 °C or -20 °C under an air-permeable or vacuum-sealed plastic film. The rancid odour developed more intensely during preservation under plastic film at -10 °C especially after 20 days of storage. The formation of unpleasant odours during storage at +8 °C of chicken fillets under a modified atmosphere (0, 2 or 4% oxygen) was faster (2 vs. 5 days) and more important (score 5 vs. 4) than that observed at +4 °C **(Pettersen et al., 2004)**. Chicken fillets stored under a modified atmosphere (70% CO_2, 30 % N_2) had higher taste and odour scores than fillets stored under air-permeable plastic film, especially after 6 days of storage at +4 °C **(Latou et al., 2014)**.

2 AIM OF THE WORK

The aim of the scientific monograph was to investigate the effect of applying different doses of flax and pumpkin pomace to feed mixture in the fattening of hybrid combination Ross 308 chickens on:

a) the final live weight of chickens of the Ross 308 hybrid combination without and after application of different doses of flax and pumpkin pomace to the feed mixture,

b) meat performance (carcass weight, giblets weight, carcase yield) of Ross 308 broiler chickens without and after application of different doses of flax and pumpkin pomace to the feed mixture,

c) the nutritional value of the carcass most valuable parts of the Ross 308 broiler (breasts, thighs) without and after application of different doses of flax and pumpkin pomace to the feed mixture,

d) sensory quality of the most valuable parts of the Ross 308 broiler chickens (breasts, thighs) without and after application of different doses of flax and pumpkin pomace to the feed mixture,

e) overall, the aim of the scientific monograph was to make a statistical evaluation of the endpoints and to recommend appropriate tested feed supplements for feeding the hybrid combination Ross 308 broiler chickens.

3 MATERIAL AND METHODS

3.1 Object of research

The object of the research was the use of flax and pumpkin pomace, which were used in complete feed mixture (FM) of broiler chickens of the hybrid combination Ross 308 in various quantities. Their effect on final live body weight, selected meat performance indicators, chemical composition, and sensory quality of the most valuable parts of the Ross 308 chickens's carcasses were investigated.

3.2 Technical realization of the experiment

3.2.1 Housing system

The experiment was realized in the Slovak University of Agriculture (SUA) in Nitra (Test Poultry Station, Kolíňany). The housing consisted of 5 boxes according to the experimental scheme.

Table 5a: Experimental scheme and dosing of feed supplements in the experiment

Group	Feed supplement	
Control C	Without feed supplement	-
1. experimental E1	Flax pomace	(20 $g.kg^{-1}$ FM)
2. experimental E2	Flax pomace	(40 $g.kg^{-1}$ FM)
3. experimental E3	Pumpkin pomace	(20 $g.kg^{-1}$ FM)
4. experimental E4	Pumpkin pomace	(40 $g.kg^{-1}$ FM)

The boxes were separated from each other by perforated mesh. 100 single-day Ross 308 chickens were placed in each box per experimental group. 43/2007 / EC. The lower layer of bedding up to a height of 7 cm consisted of wood sawdust and the upper layer of 5 cm was made of treated rolled wheat straw. Until the age of 14 days, the chickens received food from plate feeders and water from hat waterers placed on the floor. From the 15th day of age until the end of the experimental period (42nd day of fattening), the chickens received feed from tube feeders and water from buckets.

Breeding and microclimatic conditions were the same for all groups of the experiment and in accordance with the recommendations for the Ross 308 broiler chickens. Ventilation in the hall was provided by a ventilation system and ventilation windows. The light mode was automatically set in accordance with the requirements for this type of broiler chickens.

3.2.2 Feed mixtures

The complete feed mixtures were mixed in the feed company Biofeed a.s. (Slovak republic). The composition and nutritional value of the used feed mixtures are given in Tables 5b and 6.

Table 5b: Percentage composition of feed mixtures during the experiment

Component (%)	Feed mixtures	
	Starter 1. – 21. day	Grower 22. – 42. day
Corn	28.00	39.00
Wheat	34.50	30.00
Soybean meal (45% [1]NS)	31.00	26.00
Sodium bicarbonate	0.20	0.20
Calcium formate	0.80	0.80
Soy oil	3.00	1.95
Monokalcium phosphate	0.90	0.55
Calcium carbonate	0.65	0.60
Fodder salt	0.20	0.20
L-Lysine HCL	0.10	0.05
DL-Methionine	0.15	0.15
[2]Vitamine-mineral premix	0.50	0.50

[1]NS – nitrogen substances, [2] active substances in 1 kg of vitamin-mineral premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D 3 800 000 IU; niacin 12 000 mg; pantothenic acids 3 000 mg; riboflavin 1 800 mg; pyridoxin 1 200 mg; thiamn 600 mg; menadion 800 mg; ascorbic acid 50 000 mg; folic acid 400 mg; biothin 40 mg; vitamin B_{12} 10 mg; cholín 100 000 mg; betain 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg.

Table 6: Nutritional composition of used feed mixtures (g.kg^{-1})

Indicator	Starter FM (1. – 21. day)	Grower FM (22. – 42. day)
Nitrogen substances	209.68	207.00
Fiber	28.71	24.00
Lysine	11.21	12.50
Methionine	4.59	5.20
Linoliec acid	27.82	24.04
Ca	8.11	8.50
P (total)	6.03	4.00
Na	1.61	1.60
[1]ME_N (MJ.kg^{-1})	11.93	12.75

[1]ME_N – metabolized energy

3.3 Monitored indicators of the experiment

1. ***Indicators of meat performance:***

- Average live body weight (LBW) at the end of the fattening period (g),
- Carcass weight (CW),
- Giblets weight (g),
- Carcass yield (%).

2. ***Indicators of nutritional quality of muscle:***

- Chemical composition (content of water, protein, fat and cholesterol) in breast muscle (g.100 g^{-1}),
- Chemical composition (content of water, protein, fat and cholesterol) in thigh muscle (g.100 g^{-1}).

3. ***Indicators of sensory quality of meat:***

- Sensory evaluation of breast muscle (smell, taste, juiciness, tenderness – points),
- Sensory evaluation of thigh muscle (smell, taste, juiciness, tenderness – points),
- Overall sensory evaluation of breast and thigh muscle (total points).

3.4 Methods and principles for monitoring experiment indicators

3.4.1 LBW, giblets weight and carcass yield of broiler chickens at the end of the experiment

Each chicken (n = 100 pcs.group^{-1}) was weighed on KERN 440-49N scales with an accuracy of d=0.01 g after 12 hours of fasting and its live weight was determined. After determining the live weight, the chickens were selected and killed in the number of 10 pieces based on average live weight of the group without gender difference in the slaughterhouse of the Slovak University of Agriculture in Nitra.

After dissection, the carcasses were weighed to monitor the CW and the weight of the edible giblets (gizzard, neck, heart, liver) and then the relevant carcass yield was determined by calculation.

3.4.2 Chemical composition of chicken meat

Sample preparation for chemical analysis

After slaughter of the chickens, samples weighing 50 g were taken from the carcasses of broiler chickens, separately from skin and thigh muscle without skin and subcutaneous fat.

Chemical analysis of samples

Fourier transform infrared spectroscopy (FTIR), Nicolet 6700 instrument was used for chemical analysis of breast and thigh muscles. It is an analytical technique designed primarily for the identification and structural characterization of organic compounds and inorganic elements.

Samples of breast and thigh muscle were analysed for the content of water, proteins, fat, and cholesterol.

3.4.3 Sensory evaluation of the most valuable parts of carcass – breasts and thighs

We performed sensory evaluation after treatment of chicken halves by baking with the addition of 20 ml of water for 60 minutes at a temperature of 220 °C and subsequent broilingafter baking for 15 minutes. Subsequently, samples of the relevant carcass muscle

(10 breasts, 10 thighs) were taken from chicken halves and assessed anonymously by a 6-member commission with a 5-point system (smell, taste, juiciness, tenderness).

3.5 Statistical methods

The results of the experiment were statistically evaluated and calculated by analysis of variance – ANOVA using XLSTAT software (Addinsoft, New York, USA, 2021). Data are reported as arithmetic mean ± standard deviation. Statistical significance between experimental groups was calculated using the Duncan test and differences between experimental groups were considered significant at $p \leq 0.05$.

The results are processed in the form of text and tables.

4 RESULTS AND DISCUSSION

The influence of flax and pumpkin pomace as an addition to complete feed mixtures in the amount of 2 and 4% in the nutrition of chickens of the hybrid combination Ross 308 on the achieved LBW at the end of fattening, CW, giblets weight, carcass yield and meat quality. represented by the chemical composition of the carcass most valuable parts was studied.

4.1. Live body weight of the chickens at the end of fattening and selected meat performance indicators

LWB at the end of fattening and selected meat performance indicators of hybrid combination Ross 308 are presented in the Table 7 and 8.

Table 7: Average LBW of broiler chickens Ross 308 (g) at the age 42 days

Group		C	E1	E2	E3	E4	p-value
n		100	100	100	100	100	
Indicator							
LBW	$\bar{x}$	2001.20[b]	1995.20[b]	2115.70[ab]	2063.10[ab]	2211.00[a]	0.010
	SD	±137.63	±212.06	±110.39	±140.74	±100.86	

n – chickens per group; x̄ – arithmetic everage; SD – standard deviation; C – control group; E1 – 1. experimental group; E2 – 2. experimental group; E3 – 3. experimental group; E4 – 4. experimental group; different letter in the upper index means statistically significant differences between experimental groups

Table 8: Selected carcass indicators of broiler chickens Ross 308

Group		C	E1	E2	E3	E4	p-value
n		10	10	10	10	10	
Indicator							
Carcass weight	$\bar{x}$	1452.60	1371.30	1436.60	1355.10	1451.60	0.051
	SD	±99.23	±167.53	±98.21	±94.19	±62.49	
Giblets weight (g)	$\bar{x}$	143.08	143.90	148.20	138.27	143.00	0.105
	SD	±11.86	±10.10	±14.28	±10.48	±6.21	
Carcass yield (%)	$\bar{x}$	79.75[a]	75.93[b]	74.88[bd]	72.41[cd]	72.15[c]	0.001
	SD	±1.26	±3.24	±2.77	±2.13	±1.43	

n – chickens per group; x̄ – arithmetic everage; SD – standard deviation; C – control group; E1 – 1. experimental group; E2 – 2. experimental group; E3 – 3. experimental group; E4 – 4. experimental group; note: letter in the upper index mean statistically significant differences between groups

LBW is one of the basic indicators of fattening livestock, including poultry. By evaluating this indicator in Ross 308 broiler chickens fattened in the poultry test station of SUA in Nitra (Fattening station in Kolíňany), we can conclude that after the addition of flax and pumpkin pomace applied into FMs (2 and 4%), the live weight at the end of fattening increased in all experimental groups except experimental group 1 (2% addition of flax pomace), compared to the control group.

The highest live weight was recorded in experimental group 4 (2211.00 g) when the pumpkin pomace supplement was applied in an amount of 4% to FMs. LBW in this group was higher by 209.80 g compared to the control group (2001.20 g). The other experimental groups, apart from the group 1 (1995.20 g), also achieved a higher live weight at the end of fattening when flax (4%) and pumpkin pomace (2 and 4%) were applied to the FMs, namely 2115.70 g (E2), 2063.10 g (E3) and 2211 g (E4).

From a statistical point of view, we can state that significant differences ($P<0.05$) compared to the control group were recorded only with experimental group 4, to which pumpkin pomace was applied in the amount of 40 $g.kg^{-1}$ to FMs. Among the experimental groups, significant differences in LBW were achieved in broiler chickens Ross 308 ($P>0.05$) only between groups E1 (1995.20 g) and E4 (2211 g).

Feed antibiotics as growth stimulants have been commonly used for decades to support the growth performance of broiler chickens. Following the ban on their addition to feed mixtures, interest in the traceability of plant feed supplements increased and therefore properties of various plant ingredients affecting the intestinal microflora, nutrient digestibility, gastrointestinal morphology, meat performance and chicken meat quality began to be investigated **(Nkukwana et al., 2014)**. However, there is a lack of other research aimed at application of supplemental flax or pumpkin pomace and therefore we used similar research to discuss with.

The study of **Zając et al. (2020)** determined the effect of the addition of 15% camelina, flax, and sunflower seeds to iso-caloric and iso-nitrogenous diets for broiler chickens Ross 308 on the production traits and slaughter analysis parameters (among the others). Among the production parameters, we can compare to achieved LBW, carcass yield and selected edible organs weight in this research. LBW after 42 days was higher in groups that received supplements into their diet (camelina – 2254 g, flax – 2206 g, sunflower – 2174 g) compared to the control group (2064 g). Achieved carcass yield was higher compared to us – 76.1% in control group and higher (camelina – 78.2%, flax – 78.6%, sunflower – 76.9%). Birds were characterized for significantly highest gizzard weight after camelina and sunflower supplementation (34.4 and 36.8 g, respectively), while liver and heart weight were in average on a same level in experimental groups (51.1 and 12.4 g, respectively).

Zając et al. (2021) also evaluated the effect of micronized full-fat camelina inclusion, flax, or sunflower seeds in the diet for broiler chickens Ross 308 on the performance productivity, nutrient utilization, and composition of intestinal microbial populations and to assess the possibility of modification of the resistance of isolated bacteria to chemotherapeutic agents with different mechanisms of action. Compared to us, they also found a higher average final LBW after application of selected supplements. Compared to control group (2067 g), they observed significantly higher LBW namely after application of 15% micronized camelina (2238 g) and flax seeds (2220 g).

In the experiment of **Apperson and Cherian (2017)** the effect of flax seed and carbohydrase enzyme on bird performance and other indicators was evaluated. Broiler chickens RossxRoss received corn-soybean meal basal diet adjusted for 10% and 15% of flax or 10% and 15% of flax + 0.05% enzyme. Although there were no significal differences in final LBW, authors found the highest weight in the group fed with additional 15% flax and 0.05 enzymes (1628.24 g) compared to the lowest weight after supplementation with only 10% flax (1450 g) at the 40th day aof age.

Azida, Quezada and Cherian (2010) added supplemental Camelina (false flax) meal to Cobb chicks' diet in the amounts 0% (control), 2.5% (CAM2.5), 5% (CAM5), and 10% (CAM10). After feeding period of 42 days, significant difference in final LBW was observed among birds in the higher control (1958.05 g) and CAM5 (2012.48 g) treatments compared to lower LBW in CAM2.5 (1784.46 g) treatment. There were no significant differences in carcass weight, which was approximately of the same level – 1544.14 g (Control), 1507.70 g (CAM2.5), 1,488.33 g (CAM5) and 1,540.88 g (CAM10). Although these authors did not observe giblets weight as we did, they at least described liver weight as a percentage of carcass weight, that was significantly highest for the CAM2.5 diet – 3.06% and highest heart proportion in control group – 0.83%.

There is evidence that pumpkins are used traditionally and on a small scale in the feeding of several species of domestic animals such as ruminants **(Lans et al., 2007)** and equines **(Lans et al., 2006; OECD, 2016)**. Research on the use of pumpkins in animal feeding and its productivity benefits are attributed to its protein and fat content in the case of seeds, and carbohydrates, minerals, and vitamins in the case of the fruit **(Achilonu et al., 2018)**.

The number of studies regarding the use of pumpkin seeds as animal feed has been conducted in broiler chickens in which an increase in weight gain and carcass yield was observed. In this regard, **Aguilar et al. (2011)** reported greater weight, greater breast yield, and lower abdominal fat when 6% of *Cucurbita moschata* seed flour was included in the diet, while **Zinabu et al. (2019)** improved weight gain with only 1% of Cucurbita maxima seed flour included in the chicken diet. **Hajati, Hasanabadi and Waldroup et al. (2011)** showed that an addition of 5 g/kg of pumpkin seed oil to the ration does not affect the productive performance. In laying hens, there were no changes in the laying rate or the quality of the egg using pumpkin seed flour **(Martínez et al. 2010; 2012)**. In the case of turkeys, the use of 5% of seeds in their diet improved the fertility of the eggs, reduced embryonic death, and increased hatching rate **(Machebe et al., 2013)**.

A study was conducted using 200 Anak – 2000 one week old chicks to evaluate the effects of feeding varying levels of pumpkin seed meal (PSM) on the growth performance and carcass characteristics of broiler chickens. The chicks were randomly assigned to five dietary treatments consisting of four replicates of ten birds per replicate which received diet T1 (control) contained 0% pumpkin seed meal (PSM) while diets T2, T3, T4 and T5 contained 5%, 10%, 15% and 20% PSM respectively. The result of growth performance showed that final LBW results were contrary to our results with pumpkin pomace as the observed LBW in this research was significantly highest in the control group, like the 20% supplementation. Carcass yield, breast, thigh, abdominal fat, kidney, gizzard, liver, and lungs weights did not differ significantly as the levels of PSM increased in the diets. It was concluded from this study that pumpkin seed meal is a good source of crude protein and can substitute soybean meal in a broiler chicken diet up to 20% **(Wafar et al., 2017)**.

In study of **Aguilar et al. (2011)** Cobb 500 broilers reared from 1 to 49 days were distributed into four treatments to evaluate the effect of the dietary inclusion of 0, 33, 66 or 100 $g.kg^{-1}$ of squash seed meal (SSM) (*Cucurbita moschata*) on the performance, carcass yield, serum lipid profile and sensory meat quality of broilers. They found higher final LBW compared to us (due to the longer fattening period), as it varied form the significantly lowest control (2277 g) and SSM100 (2274 g) compared to SSM33 (2376 g) and SSM66 (2380 g). Carcass weight varied significantly from 1625 g (SSM100) to 1722 g (SSM33), while the subsequent carcass yield did not differ among the experimental groups (ranging from 71.50% in SSM100 to 72.38% in SSM33). Although authors did not observe giblets weight, which we could compare to, they found that liver weight did not differ among the experimental group with average weight around 50 g.

Even that there is no evidence on effect of flax and pumpkin pomace in the broilers diet on meat performance, other works were aimed at the addition of bee products or the utilization of agricultural by-products such as grape pomace and others and are discussed below.

Haščík et al. (2019) observed significant ($P<0.05$) increase after addition of 400 mg bee pollen extract per 1 kg of feed mixture plus 3.3 g probiotic (*Lactobacillus fermentum*) added to drinking water daily (2401.70 g) and increase of body weight ($P>0.05$) using the same supplementation with propolis instead of bee pollen (2358.00 g) compared to control group (2270.2 g). **Haščík et al. (2010)** found that the carcass yield of Ross 308 chickens without gender difference was slightly higher in the control group (78.69%) compared to the experimental group (78.48%) but without significant differences ($P>0.05$). By sex, the carcass yield ($P>0.05$) was slightly higher in the control group (78.78%) compared to the experimental group (78.26%). Similar total giblets weight was observed by **Haščík et al. (2015, 2019)** using bee products and probiotics (ranging from 152.08 to 162.18 g), while **Haščík et al. (2010, 2013)** found much lower giblets weight using propolis and propolis extract in the broiler chicken nutrition (117.16–140.75 g)**.**

Haščík et al. (2020) applied supplemental red grape pomace (RGP) of variety Alibernet into the complete feed mixtures of Ross 308 broiler chickens and state that, the live weight of chickens was significantly highest in the group with supplemental 3% RGP (2180.90 g) in comparison with control group (2001.20 g). The highest carcass weight described by these authors were found also after 3% supplementation (1515 g), but on the other hand significantly highest carcass yield was found in control group – 79.75%. This was probably due to the higher giblets weight after RGP supplementation in all experimental groups (1% – 158.20 g; 2% – 158.61% and 3% – 155.97 g) compared to the control group (143.08 g).

Turcu et al. (2020) found a lower live body weight of Cobb 500 chickens after application of red grape pomace (variety Merlot) ($P> 0.05$) in an amount of 3 and 6% (2937.73 g and 2995.74 g) compared to the control group (3136.67 g), which contrasts with results achieved by us. Research of **Kumanda, Mlambo and Mnisi (2019)** shows significant decrease ($P<0.05$) of body weight (2844.90 g) after high dosage of 10% untreated red grape pomace compared to control group (2957.50 g). High dosage with white grape pomace flour (20%) also did not lead to an improvement of body weight in study of **Reyes et al. (2020)**. However, higher final body weight was observed by **Turcu et al. (2019)** in Hubbard broilers supplemented with 2% grapeseed meal in comparison with control group (2719.47 vs. 2545.00 g).

Tekeli, Rustu Kutlu and Celik (2014) found the highest carcass weight after the highest used grapeseed oil supplementation (1.5%) – 1540.31 g, what was 10.16 g more than in control group (1530.15 g). However, they did not find significant differences among the groups after grapeseed oil supplementation into chicken diet. While carcass yield decreased in the groups fed with 0.5 and 1% grape seed oil supplementation (68.81 and 68.07%), carcass yield of the group fed with 1.5% grape seed oil supplementation was similar (69.80%) to that of the control group (69.83%) Markedly lower carcass weight was described by **Kumanda, Mlambo and Mnisi (2019b)**, who did not find positive effects of red grape pomace supplementation, cold carcass weight decreased in 2.5, 4.5, 5.5 and 7.5 % as followed: 1229.2, 1237.1, 1161.9 and 1153.1 g, respectively, in comparison with control group (1270.7 g). Positive effect of grape pomace on carcass yield was observed by these authors after 4.5% supplementation (72.43%) compared to control group (69.64%). **Sánchez-Roque et al. (2017)** observed the effect of different agro industrial wastes on carcass characteristics of broiler chickens. They found significantly higher carcass weight ($P<0.05$) after supplementation with ferment of coffee (1810.10 g) and milk whey (1718.2 g) in comparison with control group (1463.00 g).

4.2 Chemical composition of chicken meat

Chemical composition of breast and thigh muscle without skin and subcutaneous fat of broiler chicken hybrid combination Ross 308 is presented in Tables 8 and 9.

Table 9: Chemical composition of Ross 308 broiler chickens breast muscle (g.100 g^{-1})

Group		C	E1	E2	E3	E4	p-value
n		10	10	10	10	10	
Indicator							
Water content	$\bar{x}$	70.69^{cd}	69.96^{bc}	70.87^{d}	70.15^{bcd}	71.83^{a}	0.001
	SD	±0.83	±0.40	±0.78	±0.65	±0.62	
Protein	$\bar{x}$	23.87	23.97	23.99	24.17	24.04	0.055
	SD	±0.21	±0.44	±0.38	±0.31	±0.32	
Fat	$\bar{x}$	0.96^{ab}	0.83^{b}	1.14^{a}	1.02^{a}	1.04^{a}	0.010
	SD	±0.24	±0.15	±0.28	±0.11	±0.18	
Cholesterol	$\bar{x}$	0.041^{ab}	0.039^{ab}	0.042^{a}	0.041^{a}	0.038^{b}	0.031
	SD	±0.005	±0.005	±0.005	±0.002	±0.004	

n – chickens per group; x̄ – arithmetic everage; SD – standard deviation; C – control group; E1 – 1. experimental group; E2 – 2. experimental group; E3 – 3. experimental group; E4 – 4. experimental group; different letter in the upper index means statistically significant differences between experimental groups

The nutritional value of meat, resp. its chemical composition can be assessed based on parameters such as protein content, amino acid content, fat content, fatty acids and the content of carbohydrates, minerals, and vitamins **(Suchý et al. (2002)**.

When evaluating the chemical composition of pectoral muscle without skin and subcutaneous fat, we found the lowest water content in experimental group E1 (69.96 g.100 g^{-1}). Slightly higher values in g.100 g^{-1} of pectoral muscle were recorded in the other experimental groups and the control group of the experiment (E2 – 70.87; E3 – 70.15; E4 – 71.83; C – 70.69). Statistical evaluation of the water content in the pectoral muscle revealed significant differences ($P<0.05$) between the experimental groups C:E4, E1:E2, E1:E4, E2:E4 and E3:E4.

The highest protein content in the breast muscle (24.17 g.100 g^{-1}) was recorded in the 3rd experimental group (addition of 2% pumpkin pomace), while the other groups had approximately the same values from 23.87 g.100 g^{-1} (control group) to 24.04 g.100 g^{-1} (4th

experimental group). Statistical comparison of protein content results in the breast muscle did not reveal a significant difference (P>0.05) between the individual groups of the experiment.

The fat content in the breast muscle of the tested experiment in g.100 g^{-1} was the highest in the experimental group E2 (1.14) and lower in the other groups as well as the control group (E1 – 0.83; E3 – 1.02; E4 – 1.04; C – 0.96). An interesting finding was that in the group E2 (addition of 4% flax pomace) the fat content in the breast muscle increased compared to the group E1 (2% addition of flax pomace), where it was the lowest and when dosing pumpkin pomace (2 and 4%) there was a tendency to increase fat in the breast muscle compared to the control group. From a statistical point of view, we did not notice any significant differences between the control group and the experimental groups (P>0.05). We found significant differences (P<0.05) in the fat content in the breast muscle of chickens between the experimental groups E1: E2, E1: E3 and E1: E4.

The cholesterol content in g.100 g^{-1} in the breast muscle was lower resp. balanced in the experimental groups (E1 – 0.39; E2 – 0.42; E3 – 0.41; E4 – 0.38) compared to the control (0.41). Significant statistical differences (P<0.05) between experimental groups were found in cholesterol content between experimental groups (E2: E4; E3: E4).

Table 10: Chemical composition of Ross 308 broiler chickens thigh muscle (g.100 g^{-1})

Group		**C**	**E1**	**E2**	**E3**	**E4**	**p-value**
n		10	10	10	10	10	
Indicator							
Water content	$\bar{x}$	70.85^{a}	70.41^{ab}	70.57^{ab}	70.22^{b}	70.73^{ab}	0.033
	SD	±0.63	±0.46	±0.55	±0.44	±0.75	
Protein	$\bar{x}$	21.79	21.75	21.84	21.87	21.82	0.120
	SD	±0.14	±0.13	±0.25	±0.15	±0.17	
Fat	$\bar{x}$	2.25^{b}	2.73^{a}	2.54^{ab}	2.53^{ab}	2.75^{a}	0.008
	SD	±0.34	±0.24	±0.36	±0.37	±0.29	
Cholesterol	$\bar{x}$	0.058^{b}	0.062^{ab}	0.062^{ab}	0.062^{ab}	0.065^{a}	0.010
	SD	±0.005	±0.005	±0.004	±0.006	±0.004	

n – chickens per group; x̄ – arithmetic everage; SD – standard deviation; C – control group; E1 – 1. experimental group; E2 – 2. experimental group; E3 – 3. experimental group; E4 – 4. experimental group; different letter in the upper index means statistically significant differences between experimental groups

Table 10 shows the chemical composition of the skinless and subcutaneous fat free thigh muscle in chickens of the Ross 308 hybrid combination. The water content in g.100 g^{-1} was lowest in experimental group E3 (70.22) and slightly higher in the the control group and other experimental groups to which various additions of flax (2 and 4%) and pumpkin pomace (4%) were added (E1 – 70,41; E2 – 70,57; E4 – 70,73; K – 70,85). Statistical evaluation revealed significant differences ($P<0.05$) only between the control group and group E3 with the addition of 2% pumpkin pomace into chickens' diet.

The protein content in the experiment was on the high level in the thigh muscle of Ross 308 chickens, as values from 21.75 (E1) to 21.87 g.100 g^{-1} (E3) were found, which is close to the protein content declared in the breast muscle of chickens. Statistically, no significant differences were found between the experimental groups ($P>0.05$).

The fat content in the thigh muscle of Ross 308 chickens reached relatively low values, ranging from 2.25 g.100 g^{-1} (C) to 2.75 g.100 g^{-1} (E4). There was a tendency for a slight increase in fat content in the thigh muscle ($P<0.05$) in the experimental groups E1 and E4 compared to the control group. As the high protein content in the femoral muscle of Ross 308 chickens was found in the monitored groups of the experiments, the tendency of a slight increase in the fat content in this muscle in the experimental groups was also confirmed.

The cholesterol content in the thigh muscle of Ross 308 chickens was balanced in the monitored experimental groups of the experiment ($P>0.05$), ranging from 0.062 (E1, E2 and E3) up to 0.065 g.100 g^{-1} (E4). The lowest value of cholesterol content in the thigh muscle of Ross 308 chickens was recorded in the control group (0.058 g.100 g^{-1}) and we found a statistically significant difference only between K: E4.

The study of **Zając et al. (2020)**, aimed at the effect of the addition of 15% of camelina, flax, and sunflower seeds to iso-caloric and iso-nitrogenous diets for broiler chickens Ross 308, was also evaluating dietary value of breast and drumstick meat. Water content was higher compared to our results, ranging from 75.5% (control group) to 77.2% (sunflower) in the breast muscle and from 73.9% (control group) to 76.1% (sunflower). On the other side, these authors found lower protein content compared to our results, ranging from 20.3% (sunflower) to 21.4% (control) in the breast muscle and from 17.6% (sunflower) to 18.6% (control) in the thigh muscle. Our results on fat content in the breast muscle after flax pomace are well comparable with these authors, as they found fat content after flax supplementation 1.11%. On the other side, in thigh muscle, these authors found approximately two-times higher fat content compared to us. These authors also determined ash content, which was stable in both breast (±1.14%) and thigh muscle (±1.2%).

Apperson and Cherian (2017) also evaluated lipid and fatty acids content of breast and thigh muscle as par of their research. As above mentioned, broiler chickens RossxRoss in their experiment received corn-soybean meal basal diet adjusted for 10% and 15% of flax or 10% and 15% of flax + 0.05% enzyme. Authors found significanly highest fat content of breast muscle in group that received 10% of supplemental flax and on the other side lowest fat content was observed afper supplemental 15% flax + 0.05 enzyme. Total fat content in thigh muscle corresponded with breast muscle, as the highest observed fat content was in grup fed with additional 10% (2.23%) versus 1.70% in the groups that received 10 or 15% flax + 0.05% enzymes. Their observed results are also well comparable to ours.

Azida, Quezada and Cherian (2010) in their experiment used supplemental Camelina (false flax) meal to Cobb chicks' diet in the amounts 0% (control), 2.5% (CAM2.5), 5% (CAM5), and 10% (CAM10). They found increasing effect of this supplemenent in breast muscle (CAM2.5 – 0.77%; CAM5 – 0.72% and CAM10 – 1.06% versus 0.65% in control), while in thigh muscle values were contradictory (CAM2.5 – 1.77%; CAM5 – 1.67% and CAM10 – 2.14% versus 1.83% in control). Although our observed breast and thigh muscle content was higher, it was probably due to the other selected broiler hybrid.

The study was conducted on female Pharaoh quails aged from 7 to 20 weeks, divided into 3 dietary groups. The control group received standard feed for adult birds, whereas group I received feed containing 4% flax seed and group II received feed containing 7% flax seed. Feeding quail with feed enriched with 4% and 7% flax seed did not increase the protein content in breast muscles. There was a slight decrease in fat content and dry matter in breast muscles from birds fed with 7% flax seed feed compared to control. In leg muscles the use of flax seed in the feed resulted in a statistically significant decrease in dry matter, protein and intramuscular fat in both experimental groups. The use of 7% flax seed in feed resulted in significantly less fatty acids: C14 : 0, C16 : 0, C17 : 0, C18 : 0, and more unsaturated fatty acids: C16 : 1c, C18 : 1 n–9c, C18 : 3 n–3, C20 : 3 n–6, C22 : 5 n–6 **(Jakubowska et al., 2012)**.

Even though **Aguilar et al. (2011)** did not examine the chemical composition of broilers muscle, they discovered interesting effect of supplemental squash seed meal – the serum levels of total cholesterol, very low density and low density lipoproteins, triglycerides, glucose and atherogenic index decreased with the inclusion of 100 $g.kg^{-1}$ of supplemental squash seed meal, except for high density lipoproteins, which increased.

Although there is almost none evidence on effect of supplemental pumpkin pomace in broilers diet on chemical composition of muscle, pumpkin as whole fruit in livestock nutrition was the aim of other authors. The dietary inclusion of pumpkin seed could improve the composition of chicken meat. In this regard, **Hajati et al. (2011)** reported a decrease in plasmatic cholesterol and triglycerides of chickens fed with a diet containing 1% of pumpkin seed oil. On the other hand, **Antunovic et al. (1970)** also reported a decrease in blood cholesterol in sheep when they replaced soybean meal with up to 15% of pumpkin seed pomace in the diet, without changes in the lipid profile in the semi-membranous muscle. In dairy sheep, pumpkin seed cake increases omega-3 and branched-chain fatty acids, which could have important implications for human health **(Klir et al., 2017)**.

Regarding the seeds, **Aguilar et al. (2012)** reported that 10% of *Cucurbita maxima* seed meal on the diet of laying hens increases the content of various essential mono and polyunsaturated fatty acids. Furthermore, by increasing the pumpkin meal in the diet, the content of omega-3 fatty acids increased more than double (from 454 mg.100 g^{-1} in the control group to 1095 mg.100 g^{-1} of yolk in the treated group with a 10% of pumpkin seed flour added). Moreover, there was a 10% decrease in the cholesterol content in the egg. The previous results could contribute to improve the human diet since the consumption of cholesterol and some lipids is considered unhealthy **(Achilonu et al., 2018)**.

Despite the lack of studies on flax and pumpkin pomace supplementation, other by-products and natural substances are more frequently used feed supplement in broiler chickens's diet.

Significantly lower water content revealed **Nardoia (2016)** in broilers breast muscle after feed-addition of 4% grape skin (74.38 g.100 g^{-1}) and 4% of grape pomace (74.61 g.100 g^{-1}) compared to control group (75.35 g.100 g^{-1}). Any significant differences were described after enrichment with grape pomace supplementation of 2.5%, 5% and 7% in study of **Bennato et al. (2020)** with water content ranging around Ø 73.6 g.100 g^{-1}. Neither **Reyes et al. (2020)** did not find significant differences (in breast, resp. thigh muscle) after 20% addition of white grape pomace (73.3 and 74.0 g.100 g^{-1}, respectively) and 20% addition of red grape pomace (72.6, resp. 73.3 g.100 g^{-1}) compared to control group (73.5 and 73.1 g.100 g^{-1}, respectively). Compared to us, higher water content was discovered in study of **Gungor and Erener (2020)** after addition of raw and fermented sour cherry kernel into broiler chickens' diet, ranging from 73.03% (1% fermented sour cherry kernel) to 74.39% (2% raw sour cherry kernel) in breast muscle and from 75.62% (2% fermented sour cherry kernel) to 77.23% (1% raw sour cherry kernel) in thigh muscle.

Any significant differences were evaluated after enrichment with grape pomace supplementation of 2.5%, 5% and 7% in study of **Bennato et al. (2020)** when crude protein ranged from 23.4 g.100 g^{-1} (control group) to 24.3 g.100 g^{-1} (7% addition of grape pomace). Neither **Reyes et al. (2020)** did not find significant differences (P≥0.05) (in breast, resp. thigh muscle) after 20% addition of white grape pomace (23.8, respectively 18.3 g.100 g^{-1}) and 20% addition of red grape pomace (23.2, respectively 18.9 g.100 g^{-1}) compared to control group (23.9, respectively 19.0 g.100 g^{-1}). On the other hand, **Haščík et al. (2018)** revealed significantly highest protein content in breast muscle without any supplementation in control group (23.42 g.100 g^{-1}) compared to supplementation with humic acid and probiotic (22.49 g.100 g^{-1}). In the case of thigh muscle was measured the lowest value of crude protein content 18.70 g.100 g^{-1} in experimental group fed with addition of humic acid and probiotic *Lactobacillus fermentum* and the higher value in experimental group fed with addition growth FM with Salinomycinum sodium and coccidiostaticum (19.93 g.100 g^{-1}).

Nardoia (2016) did not find significant differences in fat content in broilers breast muscle after feed-addition of 4% grape seeds (1.97%), 4% grape skin (1.59%) and 4% of grape pomace (1.74%) compared to control group (1.71%). **Bennato et al. (2020)** found reliance in increasing fat content after enrichment with grape pomace supplementation of 2.5% (1.16 g.100 g^{-1}), 5% (1.22 g.100 g^{-1}) and 7% (1.25 g.100 g^{-1}) as we did, however these differences were not significant. **Reyes et al. (2020)** found significant differences in breast muscle after 20% addition of white grape pomace (1.6%) and 20% addition of red grape pomace (2.8%) compared to control group (0.9%). **Haščík et al. (2018)** found significantly lowest fat content after enrichment with 1% humic acid in breast muscle (0.84 g.100 g^{-1}) and in control group in thigh muscle (7.15 g.100 g^{-1}). Any significant changes were also observed after dietary supplementation with raw and fermented sour cherry kernel (1, 2 and 4%) in breast and thigh fat content **(Gungor and Erener, 2020)** or supplemental pine needles powder (0.25, 0.50, 0.75 and 1%) in breast fat content **(Ramay and Yalçın, 2020)**.

Cholesterol lowering in broiler meat (breasts and thighs) is important for human nutrition and health, in reducing the risks associated with cardiovascular diseases **(Pavlović et al., 2018)** what is unfortunately in the conflict with our results on cholesterol content in thigh muscle.

Turcu et al. (2019) observed, that after addition of grape seed meal the cholesterol level in the fat from the breast meat samples was 8.13% lower in the experimental group (0.041 g.100 g^{-1}) compared to the control group (0.044 g.100 g^{-1}) and 23.85% lower in the experimental group (0.046 g.100 g^{-1}) compared to the control group (0.061 g.100 g^{-1}) in thigh muscle. **Haščík et al. (2016)** discovered higher cholesterol content in thigh muscle in experimental groups – 113.08 mg.100 g^{-1} (probiotics addition), 0.128 mg.100 g^{-1} (propolis addition) in comparison with control group 0.121 g.100 g^{-1}. Comparable results were described by **Haščík et al. (2018)** who also did not find any differences in chicken breasts cholesterol content, ranging from 0.033 g.100 g^{-1} (experimental group P1 with addition humic acids) to 0.039 g.100 g^{-1} (experimental group P2 with addition humic acids and probiotic). In thigh muscle, the cholesterol content was significantly lowest in control group – 0.070 g.100 g^{-1} compared to 0.086 g.100 g^{-1} in experimental groups E1 and E2.

4.3. Sensory evaluation of chicken meat

Sensory evaluation of breast muscle without skin and subcutaneous fat and and thigh muscle with skin and subcutaneous fat of Ross 308 chickens after meat preparation by roasting without and after application of flax and pumpkin pomace in their nutrition is shown in Tables 11 and 12.

Table 11: Sensory evaluation of Ross 308 broiler chicken breast muscle

Group		C	E1	E2	E3	E4	p-value
n		10	10	10	10	10	
Indicator							
Smell	$\bar{x}$	3.68	3.55	3.75	3.30	3.60	0.556
	SD	±0.67	±0.44	±0.64	±0.68	±0.66	
Taste	$\bar{x}$	3.50	3.20	3.30	2.95	3.25	0.265
	SD	±0.44	±0.42	±0.68	±0.44	±0.59	
Juiciness	$\bar{x}$	3.25	2.75	3.25	2.55	3.20	0.110
	SD	±0.64	±0.49	±0.43	±0.78	±0.92	
Tenderness	$\bar{x}$	3.58^{a}	2.75^{bc}	3.30^{a}	2.45^{c}	3.20^{ab}	0.001
	SD	±0.68	±0.49	±0.42	±0.73	±0.67	
Total points	$\bar{x}$	14^{a}	12.25^{ab}	13.60^{a}	11.25^{b}	13.25^{a}	0.020
	SD	±1.38	±1.40	±1.94	±2.15	±2.63	

n – chickens per group; x̄ – arithmetic everage; SD – standard deviation; C – control group; E1 – 1. experimental group; E2 – 2. experimental group; E3 – 3. experimental group; E4 – 4. experimental group; different letter in the upper index means statistically significant differences between experimental groups

By monitoring the individual sensory quality properties of the breast muscle of broiler chickens Ross 308 in the experiment, the best smell was observed in experimental group E2 (3.75 points) and the lowest was in group E3 (3.30 points), while in the control group we reached 3.68 points. There were no statistically significant differences (P>0.05) between the examined groups of breast muscle odor.

The taste of the breast muscle was best evaluated in the control group (3.50 points) and the worst in the group E3 (2.95 points) and again we did not find statistically significant differences between the groups as with the smell (P>0.05).

The juiciness, which depends on the water and fat content in the muscle, was highest in the breast muscle in the control group and the experimental group E2 (3.25 points) and the lowest in the experimental group E3 (2.55 points). No statistically significant differences ($P<0.05$) in juiciness between the individual groups of the experiment were found.

Tenderness was highest in the control group (3.58 points) and lowest (2.45 points) in the P3 experimental group. Based on a statistical evaluation of breast muscle softness, we found significant differences between C: E1, C: E3, E1: E2 and E2: E3.

In terms of the overall sensory evaluation of the breast muscle of the Ross 308 broiler chickens after baking, we found the highest value in the control group (14.00 points) and the lowest in the experimental group E3 (11.25 points). From a statistical point of view, we can state that the values in the individual groups of the experiment were not balanced, and significant statistical differences were recorded between the groups C: E3, E2: E3 and E3: E4.

Table 12: Sensory evaluation of Ross 308 broiler chicken breast muscle

Group		**C**	**E1**	**E2**	**E3**	**E4**	**p-value**
n		10	10	10	10	10	
Indicator							
Smell	$\bar{x}$	4	3.65	4.25	3.63	3.85	0.329
	SD	±0.85	±0.67	±0.64	±0.74	±0.85	
Taste	$\bar{x}$	3.93	3.55	3.90	3.65	3.75	0.749
	SD	±0.90	±0.50	±0.62	±0.63	±0.92	
Juiciness	$\bar{x}$	3.85[a]	3.20[b]	3.95[a]	3.85[a]	4[a]	0.025
	SD	±0.67	±0.68	±0.44	±0.53	±0.58	
Tenderness	$\bar{x}$	3.80	3.20	3.95	3.70	4	0.057
	SD	±0.89	±0.75	±0.50	±0.42	±0.53	
Total points	$\bar{x}$	15.56	13.60	16.05	14.83	15.60	0.142
	SD	±2.93	±2.28	±1.40	±1.58	±2.68	

n – chickens per group; x̄ – arithmetic everage; SD – standard deviation; C – control group; E1 – 1. experimental group; E2 – 2. experimental group; E3 – 3. experimental group; E4 – 4. experimental group; different letter in the upper index means statistically significant differences between experimental groups

The smell of the thigh muscle reached 4.00 points in the control group, but the highest score was recorded in the experimental group E2 (4.25 points) and the lowest in the experimental group E3 (3.63 points). Among these groups in this indicator no statistically significant differences were found (P>0.05).

The scoring for the thigh muscle taste ranged from 3.55 points (experimental group E1) to 3.93 points (control group). Statistically significant differences between the experimental groups in taste were not found (P>0.05).

The highest evaluation of thigh muscle juiciness was found in group E4 (4.00 points) and the lowest in experimental group E1 (3.20 points), and among the groups of experiment C: E1, E1: E2, E1: E3 and E1: E4 were found statistically significant differences (P>0.05).

The thigh muscle tenderness between the groups of experiment was balanced, therefore no statistically significant differences were achieved (P>0.05) and the thigh muscle tenderness was perceived best in the experimental group E4 (4.00 points) and the worst in the experimental group E1 (3.20 points).

In terms of overall sensory assessment of the thigh muscle, we found that the highest score was in the experimental group E2 (16.05 points) and the lowest in the tested experimental group E1 (13.60 points), but among the experimental groups (C, E1, E2, E3, E4) no statistically significant difference was found (P>0.05).

In general, as we confirmed in our experiment, the fineness of the thigh muscle was higher than the pectoral muscle, which is in line with the results of published research by **Haščík et al. (2013)** and **Haščík et al. (2014)** because the thigh muscles contain more internal fat and blood capillaries.

Cucurbita spp. seeds and flaxseeds are generally known for high concentrations of polyunsaturated fatty acids. However, according to **Shukla and Perkins (1998)**, linoleic and alfa-linolenic acids are the most resistant to oxidation among the polyunsaturated fatty acids but are more susceptible as compared to saturated and monounsaturated fatty acids. The high susceptibility of flaxseed to oxidation may be related to their high alfa-linolenic, docosahexaenoic and eicosapentaenoic acid content, which are comparatively lower in *Cucurbita* spp. seeds. This susceptibility to oxidation and subsequent undesirable odours can be then present in products as meat.

The results of sensory evaluation of cooked meat and broth in study of **Jakubowska et al. (2012)** after application of supplemental flaxseed into quails diet showed, that in most cases there were no differences in the sensory appeal of the meat or broth between the control and experimental groups. In several samples, the meat and broth in groups I and II had a distinct taste and smell of fat, which affected the average assessment of the flavour and aroma in these groups. The deviations in flavour and aroma may have been associated with a greater share of unsaturated fatty acids in the groups receiving flax seed, which is consistent with the findings of **Van Elswyk (1997)** regarding the impact of the increased content of unsaturated fatty acids on the sensory characteristics of meat.

Panel members in study of **Aguilar et al. (2011)** did not detect any differences in the sensory quality of the breast and thighs of broilers Cobb fed with or without supplemental squash seed meal in the amounts 33, 66 and 100 g.1000 g^{-1} of feed mixture. They gave same points for every indicator (aroma, flavor, tenderness, and color) and did not describe no abnormalities in any used supplementation. This conflict with our results, as members of our sensory panel marked as the best control, with slightly lower points score after addition of supplemental flax and pumpkin pomace.

Although there is almost none evidence on direct application of supplemental pumpkin pomace into broilers diet and its effect on meat sensory quality, pumpkin as whole fruit could find utilization in other poultry – namely in laying hens' product, eggs. Because of its carotene content **(Kim et al., 2012)**, the pulp and peel of pumpkins could be used in the diet of laying hens. The color of the yolk is a characteristic associated with the quality and freshness of the egg according to consumers. The color depends on the accumulation of carotenoids, molecules synthesized by higher plants, algae, bacteria, and fungi, and which birds obtain from the diet, either in a natural form from ingredients that contain them or by a synthetic source. The carotenoids mostly used in the yolk pigmentation are xanthophylls **(Nys, 2000)**. Additionally, the use of pumpkin seed meal, up to 100g/kg, in the diet of laying hens does not confer unpleasant flavors to the egg, an efect that has been reported when using meals of other seeds such as flax **(Aguilar et al., 2010)**.

CONCLUSION

In summary, it can be concluded that fruit and vegetable processing industries generate millions of tonnes of waste every year causing serious environmental problems if not properly handled. These by-products are an excellent source of bioactive compounds including carbohydrates, lignin, protein, fat and phenolics, among others. The direct extraction of these compounds or/and their transformation into high value-added products, not only mitigates the environmental issues but also improves the sustainability and economic competitiveness of the food industry.

Pumpkin and flax have benefits in the productive performance of livestock when they are part of the diet. Additionally, due to the high content of antioxidants and fatty acids present in the fruit and/or seeds, respectively, some of the characteristics of meat are improved, contributing to the human access to a healthier diet. The idea, in any case, is not the harvest of flaxseed and pumpkins for animal feed but to use fruits not suitable for human consumption or the waste generated after harvest or post-processing. Thus, the environmental impact of livestock production is reduced, contributing to the sustainability of animal production systems and to cover the demand for food of animal origin, which impacts the physical and mental health of the human being.

The following conclusions were reached by applying different doses of flax and pumpkin pomace to the complete feed mixtures of the chickens Ross 308 hybrid combination:

- LBW of chickens at the end of fattening (42 days) was higher in almost all experimental groups to which different doses of flax and pumpkin pomace were added compared to the control group to which these supplements were not added

- the significantly highest ($P<0.05$) LBW compared to the control group (2001.20 g) was in the group after application of 4% pumpkin pomace to the complete feed mixture (2211 g),

- the carcass weight of Ross 308 chickens was around 1400 g, the highest being in the control group (1452.60 g), but these differences were not significant ($P>0.05$),

- giblets weight was not demonstrably affected witd used feed supplements, it ranged from 138.27 g in group E3 to 148.20 g in group E2, without significant differences rozdielov ($P>0,05$),

- unfortunately, the carcass yield as an important economic parameter was negatively affected by the selected additions to the complete feed mixture, significantly highest carcass yield was in the control group (79.75%),

- carcass yield, although significantly lower ($P<0.05$) in the experimental groups with the addition of flax and pumpkin pomace, did not fall below the standarded carcass yield – 72%,

- the chemical composition of the most valuable carcasses of Ross 308 chickens was only minimally affected in numerical values by the application of supplemental pomaces,

- significantly highest ($P<0.05$) water content in the E4 group (71.83%), fat content in the E2 group (1.14%) and cholesterol content in the E2 group (0.042%) were observed in the pectoral muscle,

- similarly, significant differences in water, fat and cholesterol content were observed in the thigh muscle ($P<0.05$); the highest water content was in the control group (70.85%), fat in the E4 group (2.75%) and cholesterol also in the E4 group (0.065%),

- although we found slight variations between the groups in the sensory evaluation, we can ultimately state that the meat from the control group and after supplementary pumpkin pomace was better evaluated,

- from the sensory evaluation of breast muscle of Ross 308 chickens we can state that the smell, taste, and juiciness of meat after and without the application of pomaces was balanced, significant differences ($P<0.05$) were observed in tenderness, when the control group received the most points (3.58) as well as in the overall evaluation (14 points),

- by sensory evaluation of the thigh muscle, we can state that significant differences ($P<0.05$) only in the juiciness, when the highest score was obtained in meat from group E4 (4 points)

- based on the overall evaluation, the addition of 4% pumpkin pomace proved to be the best both in terms of meat performance and the evaluated chemical and sensory indicators of Ross 308 chickens meat, either in comparison with the control group or with other tested supplements.

However, there is very little or no similar research on the application of flax and pumpkin pomace in chicken nutrition. Therefore, based on the results, we recommend continuing experiments with the addition of similar by-products in chickens of different hybrid combinations and continuing to examine the possible replacement of antibiotics and growth promoters with these alternatives, which are still used in chicken feed nutrition worldwide after their ban.

Abstrakt v slovenskom jazyku

Cieľom vedeckej monografie bolo skúmanie vplyvu aplikácie ľanových a tekvicových výliskov v množstve 2 a 4 % do kompletných kŕmnych zmesí vo výkrme hybridnej kombinácie kurčiat Ross 308. Na začiatku pokusu boli jednodňové kurčatá hybridnej kombinácie Ross 308 umiestnené do boxov po 100 kusov, kŕmené a napájané boli systémom *ad libidum*, pričom v pokusných skupinách na rozdiel od kontrolnej (C) im boli do kompletnej kŕmnej zmesi pridané ľanové (E1 a E2) a tekvicové výlisky (E3 a E4) v uvedených percentách. Vo vedeckej monografii bol sledovaný vplyv vybraných suplementov na finálnu živú hmotnosť kurčiat, ukazovatele mäsovej úžitkvosti (hmotnosť jatočného tela, hmotnosť drobkov a jatočná výťažnosť), chemické zloženie prsnej a stehennej svaloviny a senzorické posúdenie prsnej a stehennej svaloviny. Preukazne najvyššia ($P<0,05$) živá hmotnosť (2211 g) oproti kontrolnej skupine (2001,20 g) bola v skupine E4 po aplikácii 4 % tekvicových výliskov do kompletnej kŕmnej zmesi. Hmotnosť jatočne opracovaného tela kurčiat Ross 308 bola okolo 1400 g, pričom najvyššia bola v kontrolnej skupine (1452,60 g), tieto rozdiely však neboli preukazné ($P>0,05$). Hmotnosť drobkov nebola zvolenými kŕmnymi doplnkami výrazne ovplyvnená, nakoľko bola od 138,27 g v skupine E3 do 148,20 g v skupine E2; bez významných štatistických rozdielov medzi skupinami experimentu ($P>0,05$). Jatočná výťažnosť ako dôležitý ekonomický parameter bola zvolenými prídavkami do kompletnej kŕmnej zmesi negatívne ovplyvnená, preukazne najvyššia jatočná výťažnosť bola v kontrolnej skupine (79,75 %). Chemické zloženie najcennejších častí jatočného tela kurčiat Ross 308 aplikáciou ľanových a tekvicových výliskov bolo v číselných hodnotách ovplyvnené len minimálne, v prsnej svalovine bol pozorovaný preukazne ($P<0,05$) najvyšší obsah vody v skupine E4 (71,83 %), tuku v skupine E2 (1,14 %) a cholesterolu v skupine E2 (0,042 %). Podobne aj v stehennej svalovine boli pozorované preukazné rozdiely ($P<0,05$); najvyšší obsah vody bol v kontrolnej skupine (70,85 %), tuku v skupine E4 (2,75 %) a cholesterolu v skupine E4 (0.065 %). Pri senzorickom hodnotení sme pozorovali síce len mierne odchýlky medzi skupinami, v konečnom dôsledku môžeme konštatovať, že najvyššie bolo hodnotené mäso z kurčiat v kontrolnej skupine a v skupine E4. Na základe celkového vyhodnotenia sa ako najlepší prejavil prídavok 4 % tekvicových výliskov tak z hľadiska dosahovanej mäsovej úžitkovosti ako aj hodnotených chemických a senzorických ukazovateľov kvality mäsa kurčiat Ross 308, či už v porovnaní s kontrolnou skupinou alebo s inými preverovanými suplementmi v pokusných skupinách.

Kľúčové slová: výživa, brojlerové kurčatá, úžitkovosť, kvalita mäsa, výlisky, ľan, tekvica

Abstract in english

The aim of the monograph was to investigate the effect of application flax and pumpkin pomace in the amount of 2 and 4% into complete feed mixtures in fattening hybrid combination of broiler chickens Ross 308. At the beginning of the experiment, one-day-old chickens hybrid combination Ross 308 were placed in boxes of 100 pieces. They were fed and watered ad libidum. In the experimental groups, in contrast to the control (C), flax (E1 and E2) and pumpkin pomace (E3 and E4) in the indicated percentages were added to the complete feed mixture. The influence of selected supplements on the final LBW of chickens, post-slaughter indicators of chickens (carcass weight, giblets weight and carcass yield), chemical composition of breast and thigh muscle and sensory evaluation of breast and thigh muscle were studied. The significantly highest ($P<0.05$) live weight (2211 g) compared to the control group (2001.20 g) was in the group E4 after application of 4% pumpkin pomace to the complete feed mixture. The carcass weight of Ross 308 chickens was around 1400 g, with the highest in the control group (1452.60 g), but these differences were not significant ($P>0.05$). The weight of the giblets was not significantly affected by the chosen feed supplements, as it ranged from 138.27 g in group E3 to 148.20 g in group E2; without significant statistical differences ($P>0.05$). Carcass yield as an important economic parameter was negatively affected by the selected additions to the complete feed mixture, significantly highest carcass yield was in the control group (79.75%). The chemical composition of the most valuable parts of the Ross 308 chicken carcass with the application of flax and pumpkin pomace was only minimally affected in numerical values. In the pectoral muscle, the highest water content was observed ($P<0.05$) in group E4 (71.83%), fat in group E2 (1.14%) and cholesterol also in group E2 (0.042%). Similarly, demonstrable differences were observed in the thigh muscle ($P<0.05$); the highest water content was in the control group (70.85%), fat in the E4 group (2.75%) and cholesterol also in the E4 group (0.065%). Although we observed slight differences between the groups in the sensory evaluation, in the end we can state that the meat from the chickens in the control group and in the E4 group was better evaluated. Based on the overall evaluation, the addition of 4% pumpkin pomace proved to be the best both in terms of performance and evaluated chemical and sensory indicators of Ross 308 chicken meat, either in comparison with the control group or with other tested supplements.

Keywords: nutrition, broiler chickens, meat performance, meat quality, pomace, flax, pumpkin

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Authors: Peter Haščík, Matej Čech, Juraj Čuboň, Marek Bobko, Miroslava Kačániová, Henrieta Arpášová

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