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Effect of different feed supplements in the diet of Ross 308 broiler chickens on formation of abdominal fat

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Name: Effect of different feed supplements in the diet of Ross 308 broiler chickens on formation of abdominal fat

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INTRODUCTION

Poultry meat is among the most popular and at the same time the most produced in the world. This is mainly due to its nutritional quality, as it is a rich source of high-quality proteins and at the same time contains less fat compared to pork and beef. Poultry breeding is also undemanding. The high consumption of poultry meat is also since, unlike other types of meat, there are no cultural-religious restrictions on its consumption. From a culinary point of view, it is meat that usually does not need a long heat treatment and can be used to make various types of products and dishes.

Among all types of poultry, broiler chickens stand out, as they have the great feature of being able to reach the ideal slaughter parameters within six weeks under optimal conditions. At the same time, chicken meat is the softest and, together with turkey, contains the least fat and the most protein. It is one of the best sources of animal protein in the world due to its availability and relatively low price. That is why the production of chicken covers most of the other types of poultry.

At the beginning of the 21st century, certain problems arose in the field of animal production research in the countries of the European Union, which was mainly related to the ban on the use of feed antibiotics and components of animal origin in feed mixtures. For the purposes of increasing the safety of the food chain and the quality of animal products, the search for new alternative substances such as plant extracts, probiotics, prebiotics, etc., which would replace feed antibiotics and, like them, would stimulate growth and positively support the physiological functions of the organism, but would not have negative the effects due to which feed antibiotics were banned, especially the presence of residues in products intended for human consumption and the associated possibility of resistance of pathogenic bacteria to commonly administered antibiotics. These alternative supplements should also replace the protective function of antibiotics, while they would not cause the mentioned resistance of pathogenic bacteria. By applying them, we would like to achieve a good overall health condition of the animals, their high production capacity, and the production of health-safe food.

Enriching the feed ration of animals, including poultry, with natural substances from various feed supplements is suitable as prevention of health in individual stages of their life. In poultry, they can support overall viability, can increase productivity itself, manage stressful situations that occur in poultry flocks during fattening and, ultimately, can reduce heat stress in the hot summer months. The application of appropriate feed supplements is also associated with support in the treatment of infectious diseases.

Various natural feed supplements have a certain influence on the economically significant slaughter efficiency indicators of broiler chickens, especially such as live weight, carcass weight and the proportion of valuable meaty parts (breasts and thighs), respectively. their quality expressed by chemical composition and stability during storage and processing. One of the less monitored, but important indicators is the formation of abdominal fat. Its weight within the chicken's body may be only tens of grams, but it is an important by-product in the mass processing of broiler chickens. Although in small farms this by-product is normally processed into edible chicken fat, in large-scale production it represents unwanted waste. Natural feed supplements in the nutrition of chickens also affect the production of abdominal fat, and therefore the aim of this scientific monograph was to examine their influence on the production of abdominal fat.

1 LITERATURE REVIEW

1.1 Current state of meat and poultry production in the world and Slovak republic

Global meat production is projected to expand by nearly 44 million tons by 2030, reaching 373 million tons based on higher profitability, especially in the first years of the outlook period as meat prices rebound post-COVID19 (OECD/FAO, 2021). It is forecasted to reach 361 million tons (carcass weight equivalent) in 2022, expanding by 1.4% in 2022, albeit at a slower pace than the 4.5% growth realized in 2021. The expansion is driven mainly by a steep growth in meat output foreseen in China and notable increases in Brazil, Australia and Viet Nam, to be partly offset by anticipated declines in the European Union, the United States of America, Canada, the Islamic Republic of Iran and Argentina (FAO, 2022).

In China, overall meat production is forecast to rise to 96 million tons, growing by 4.4% year-on-year. This growth will be principally driven by a projected expansion in pig meat production by 8% to 58 million tons, exceeding the output level before the dramatic spread of the African swine fever (ASF) virus in 2018. Brazil is anticipated to increase its meat production, benefiting from disease-free status across major meat production systems and a surge in global demand, although escalating production costs and possible margin squeezes could constrain production expansion. Increased availability of competitively priced slaughter cattle following a herd-rebuilding phase and improved labor market conditions are expected to support bovine and ovine meat production expansion in Australia (FAO, 2022).

By contrast, the limited availability of slaughter-ready cattle, lower herd inventories, widespread animal diseases, and smaller profit margins could reduce meat output or slow growth in several leading producers, including the European Union and the United States (FAO, 2022).

World trade in meat and meat products is forecasted to reach 42 million tons (carcass weight equivalent) in 2022, marking the slowest growth in the last seven years. Moderate import expansions in several countries, including the United States and the United Kingdom of Great Britain and Northern Ireland, are likely to be partially offset by a steep decline in imports by China, among others.

International meat prices have been on an upward trend since October 2020, reaching an all-time high in May 2022, reflecting tight supplies from leading exporting countries amid robust global import demand (FAO, 2022). Factors as ongoing war in Ukraine and escalating energy crisis will probably rise already high prices of meat and result into lower demand of final consumers.

Global poultry meat output is forecast to reach 139 million tons in 2022, growing at a slow pace of 0.8%, as anticipated increases in the Americas, Asia, Africa and Oceania are likely to be offset by declines foreseen in Europe. At the country level, Brazil, the Russian Federation, Türkiye, the United States and Mexico are likely to register significant volume gains; however, these will be offset by anticipated declines in China and the Islamic Republic of Iran (FAO, 2022).

A surge is expected in the global demand for supplies of meat from Brazil due to disruptions to meat exports from Ukraine, coupled with constraints to exports from other leading meat producers, mostly stemming from HPAI outbreaks and squeezed profit margins. Poultry meat production in the Russian Federation is likely to rebound from a two-year contraction, helped by the increased availability of coarse grains. High foreign demand, especially from the Middle East, is expected to sustain production expansion in Türkiye. Poultry meat production in the United States is likely to be fractionally higher in 2022, reflecting a possible increase in production efficiency to counter high feed grain prices, moderate demand growth due to rising retail prices, and the impact of HPAI outbreaks (FAO, 2022).

By contrast, China’s poultry meat production is forecasted to drop, given the high probability of consumers switching back to pig meat – the preferred meat option for many consumers – with the sector’s ongoing production rebound. Meanwhile, shortages and high world prices of feed, and lower demand due to rising poultry meat prices, are likely to reduce output in the Islamic Republic of Iran (FAO, 2022). Comparison of main poultry meat production worldwide and EU 27 is shown in the Table 1; current state in Slovak republic is discussed below.

Table 1: Production of selected poultry meat in the world and EU 27 in term of fresh / chilled carcass weight (million t) (FAOSTAT, 2022)

Meat type	Region	2017	2018	2019	2020
Chicken	World	111.767	115.628	118.617	119.505
	EU 27	10.111	10.641	10.894	11.037

Turkey	World	5.887	6.024	5.961	5.993
	EU 27	1.764	1.880	1.884	1.947
Duck	World	4.632	4.929	5.013	4.998
	EU 27	0.378	0.473	0.456	0.393
Geese	World	2.672	2.793	2.775	2.842
	EU 27	0.056	0.068	0.044	0.048

According to the Statistical Office of the Slovak Republic, the number of poultry in agricultural entities in Slovakia decreased by 2.3% year-on-year to 10364.5 thousand pieces. The number of laying hens decreased by 4.8% to 3096.1 thousand pcs. As of 31.12 2021, less poultry was sold on the agri-food market by 5.1% (5389 tons), i.e., 100581 tons. For the whole year, 45257 thousand pieces of live poultry were sold for processing, trade, and export, which meant a decrease in sales in pieces compared to the previous year by 5.7% (2733 thousand pieces). In 2021, slaughterhouses in the Slovak Republic processed 77321.9 t of poultry carcass weight, which is 4320.6 t more than in 2020. The average cooled carcass weight of poultry only increased from 2.21 to 2.22 kg year-on-year (**Repka, 2022**).

Meat consumption has been shifting towards poultry. In lower income developing countries this reflects the lower price of poultry as compared to other meats, while in high-income countries this indicates an increased preference for white meats which are more convenient to prepare and perceived as a healthier food choice. Globally, poultry meat is expected to represent 41% of all the protein from meat sources in 2030, an increase of 2% when compared to the base period. The global shares of other meat products are lower: beef (20%), pigmeat (34%), and sheep meat (5%). By the end of 2nd decade of 21st century, per capita is projected to increase 0.3% p.a. to 35.4 kg in retail weight equivalent. Over one-half of this increase is due to higher per capita consumption of poultry meat (**OECD/FAO, 2021**).

Domestic consumption in Slovakia decreased by 9.3% to 127.4 thousand tons, which means a reduction of consumption by 9% to 23.4 kg per year (not including consumption of offal). Self-sufficiency in poultry meat production reached 74.1% in 2021 (**Repka, 2022**). A detailed comparison of the consumption of individual types of meat in Slovakia is shown in Table 2.

Table 2: Meat consumption in Slovak republic (kg.year.person⁻¹) (**Repka, 2022**)

Meat type	2016	2017	2018	2019	2020	2021*
Poultry	16.9	20.2	22.2	26.9	25.6	23.6
Beef and veal	4.8	5.2	5.2	5.2	5.3	5.7
Pork	35.4	35.9	35.4	35.7	35.5	39.0
All	58.4	62.8	64.3	69.3	69.9	70.0

Source: Statistical Office of Slovak republic

1.2 Breeding and nutrition of broiler chickens

1.2.1 Breeding conditions of broiler chickens

Due to the ongoing integration of many production-related disciplines such poultry health, nutrition, breeding, husbandry, and understanding of chicken products, the poultry industry has expanded and improved in recent years. A broiler body weight of 1.8 kg can be achieved with modern broiler production techniques by consuming 3.2 kg of feed in 35 days without the addition of any antibiotics to the diet (**Diarra and Malouin, 2014**).

The broiler rearing system has a significant impact on the comfort, health, and productivity of the bird. Broilers are typically housed on litter systems in the EU. In Europe, the typical broiler houses lack windows and require forced ventilation. The used litter is typically totally removed under traditional production processes in Europe after each batch, and the farm is cleaned and sanitized. The ground is then covered with a litter layer that is around 40 cm thick (**Hui and Guerrero-Legarreta, 2010**).

Ventilation is used in the open-sided houses to lower humidity and gas levels. A curtain system automatically regulates the ventilation rate. All broilers are raised on a straw, wood shavings, peat, and paper with unlimited access to water. Wet trash is a serious issue since it can cause hock burns, contact dermatitis, breast blisters, and parasitic infestation. It's crucial to have sufficient ventilation, especially in humid locations, to prevent moist litter. Since broiler chickens typically interact with litter for the entirety of their lives, the quality of the litter is crucial to their welfare. In contemporary broiler production, poor litter quality is acknowledged as a welfare issue (**Hui and Guerrero-Legarreta, 2010**).

The drinkers and feeders, which will be available throughout the fattening period, will take a few days for the chickens to acclimate. As a result, there must be enough feeders and drinkers in the hallways. The removal of existing things from the hall or the addition of new ones throughout the fattening stage has a detrimental impact on the stress of the chickens as well (**Brouček, Benková and Šoch, 2011**).

Broiler chickens have feeding habits. They spend between 30% and 50% of their time feeding, therefore it is also a crucial trait. Every day, they need between 14000 and 15000 nibbles on food or other items, in addition to raking. Disabling these behaviors results in behavioral disorders that are similar to one another. The size and mechanical characteristics of the feed have only been genetically imprinted in the chicken, so while it may eat immediately after hatching, it has no idea what kind of feed it is. This is progressively understood, and over time, less inedible material is consumed. The consumption of water is comparable. Instead, the chicken draws attention due to its physical characteristics, such as bubbles, grime, and brilliance. The drinking reaction is displayed after wetting the beak, including a head lift and swallowing. Because of this, it's crucial that chicks have easy access to feeders and drinkers and little to no access to objects that aren't edible after hatching. Normally, chickens have enough food to meet both their nutritional and energetic needs. They receive meals frequently and in tiny amounts. Broilers tend to consume more food after a prolonged fast. Greater than hunger, synchronized feed intake is significant in broiler chickens. This indicates that each chicken typically adjusts its feed intake to match the needs of the entire flock (**Tůmová, 2012**).

It has been demonstrated that nipple drinkers, whether they have drip cups or not, use less water and cause less water splashing than conventional bell-type drinkers, which lowers the moisture content of the litter and improves the sanitary quality of the litter. The environment of broilers is typically devoid of anything but litter, feeders, and drinkers (**Hui and Guerrero-Legarreta, 2010**).

Typically, feed and water are provided automatically and as needed. Standard broilers receive 3 different feeds throughout their typical 6-week growth period: The first week's starter has roughly 23% crude proteins (CP) and 12 – 13 MJ ME.kg⁻¹; the second through fourth weeks' growers have roughly 22% CP and 13 – 13.2 MJ ME.kg⁻¹; and the fifth week's finisher contains 21% CP and 13 – 13.32 MJ ME.kg⁻¹. If it doesn't contain coccidiostats, the grower diet can be given from two weeks till the end of the growing season. The typical broiler rations are pelleted. Compared to mash, this improves feed uptake and decreases meal choice and feed waste.

When the early growth rate needs to be slowed down to treat limb abnormalities, the beginning diet may be fed as mash (**Hui and Guerrero-Legarreta, 2010; Lichovniková, 2012**).

Of all the environmental elements, light might be the most important for broilers. The bird can establish rhythmicity and synchronize numerous vital processes with the help of light, including body temperature and several metabolic processes that aid in feeding and digestion. Equally significant is the stimulation of numerous hormone secretion patterns by light, which in turn affects the maturation, growth, feed conversion ratio (FCR), carcass quality, and reproductive processes in animals (**Abreu et al., 2011**).

Currently, poultry producers have access to a wide range of lighting programs (wave length, intensity, and duration) and devices, each of which has unique properties and applications for raising poultry (**Olanrewaju et al., 2006**). Before beginning the lighting program, one-day-old chicks are typically kept in constant light for the first two or three days. According to **Rutz and Bermudez (2004)**, continuous photoperiods comprise illumination schedules that are both fully continuous (24 Light: 0 Dark) and practically continuous (23 Light: 1 Dark, 16 Light: 8 Dark). While intermittent lighting better synchronizes feed intake with the passage of feed through the digestive tract of broilers, long duration continuous lighting programs enable consistent access to feed throughout the entire day, resulting in maximum feed intake and weight gain by stimulating feed intake during regular periods.

Temperature is another important factor in raising poultry. The major purpose of the structure is to safeguard birds from bad weather. As broilers are extremely sensitive to high temperatures, the two main issues are cold and hot weather. During the first few weeks of life, chicks require more heat (**Bilal et al., 2014**).

The chicks are observed to determine the final temperature setting because clustering under the brooder suggests a low temperature while avoiding the brooders indicates an elevated temperature. The advantage of zonal heating systems is that the young chick can place itself within the temperature gradient according to its needs (**Daghir, 2009; Hui and Guerrero-Legarreta, 2010**).

Tüller (1999) recommended that newly hatched chicks need ambient temperatures between 32 and 35 °C. The temperature above the litter should be regulated to 35 °C when whole-room heating is offered. At the end of the first week of age, the temperature will progressively drop to 32 °C, and in the third week, it will drop to 26 °C.

Depending on the cost of energy input and the climate, different EU nations utilize different amounts of whole room heating. Zonal brooder systems, which are powered by gas or

electricity, are typically used to generate heat. At the time of placement, the room temperature should not be lower than 25 °C and the temperature under the brooder should be 35 °C (**Daghir, 2009; Hui and Guerrero-Legarreta, 2010**).

1.2.2 Nutrition of broiler chickens

According to **Aviagen (2014a)**, the main factor affecting broiler productivity, profitability, and welfare is nutrition. To maximize the impact on health and growth, the nutritional components of feeds for chickens raised intensively are continuously modified (**Allen et al., 2013**). Depending on the species, age, and kind of production i.e., whether the birds are raised for meat production or egg production various nutrients are needed by birds (**Ravindran, 2013**).

It can be challenging to provide enough nutrients to meet the needs for the highest possible poultry output. This is because feed and feed ingredients are expensive and scarce. Producers should carefully consider the needs of each species because feed makes up 65 – 70% of the cost of production (**Hui and Guerrero Legarreta, 2010**).

Because some nutrient requirements typically energy and critical amino acids must be oversupplied to make up for the limiting nutrients in the feed, it is not economically possible to provide just the proper amount of food to meet needs. It takes specialized nutrition knowledge to balance these components to create an ideal diet for fowl (**Hui and Guerrero-Legarreta, 2010**).

Fresh and high-quality feed materials, both in terms of physical quality and nutrient digestibility, should be used in broiler diets. Wheat, maize, soya, full-fat soya, sunflower meal, rapeseed meal, oils and fat, limestone, phosphate, salt, sodium bicarbonate, minerals and vitamins, as well as other additions such enzymes and mycotoxin binders, are the key components of broiler diets (**Aviagen, 2014a**).

Energy

Energy is needed by broilers for tissue activity, growth, and maintenance (**Aviagen, 2014a**). The needs for bodily upkeep, which take precedence over production, are influenced by the bird's state of health, degree of mobility, and rate of heat loss. So, unless it is restricted by either gut fill or other physiological constraints, feed intake will rise as dietary energy content falls (**Ferket and Gernat, 2006**).

Grains, grain byproducts, animal and vegetable fats and oils make up the majority of the calories in poultry diets (**Gillespie and Flanders, 2010**). Feed formulation should adopt a method based on available energy because poultry can obtain energy from simple carbs, fat, and protein but cannot digest and use some complex carbohydrates, such as fiber (**Summers, 2000; Ravindran, 2013**).

Although protein is not typically considered a source of nutritional energy, it can help a bird meet its energy needs if fat and carbohydrates are scarce. However, it is important to limit the amount of energy that is obtained through dietary protein. As a result of the nutrients' carbon, hydrogen, and oxygen content, they can be used by the body as a source of energy (**Summers, 2000; Ravindran, 2013**).

As this is the energy available to the broiler, dietary energy levels are reported in MJ.kg⁻¹ (megajoules) or kcal.kg⁻¹ (kilocalories) of metabolizable energy (ME) (**Aviagen, 2014a**). The traditional metric for determining the amount of energy present in feed ingredients and the needs of chickens is called "metabolizable energy." This accounts for the energy losses found in pee and feces (**Ravindran, 2013**).

Fats contain nine calories per gram, compared to four for proteins and four for carbohydrates. As a result, it is typically required to include a source of fat in poultry diets when creating high energy diets (**Summers, 2000**).

The key element affecting feed consumption and diet cost is dietary energy level since the greater the amount of energy, the more expensive the diet and typically the lower the feed consumption in relation to gain (**Summers, 2000**). As a result, when creating realistic diets for poultry, the dietary energy level is frequently employed as the starting point (**Ravindran, 2013; Fouad and El-Senousey, 2014**).

When given unlimited access to food, chickens usually consume enough to meet their daily caloric needs. In comparison to chickens fed high-energy diets, those fed low-energy diets will consume more feed. To ensure that birds ingest the appropriate number of needed nutrients, the amount of required nutrients in a chicken ration must be regulated in relation to the level of energy in the ration. Because the birds would consume less of the ration daily in high-energy diets, the concentrations of nutrients must be raised. Low-energy meals must have lower nutritional concentrations because birds will consume more of them each day. When compared to low-energy feeds, high-energy rations typically result in a higher efficiency in the conversion of feed to meat or eggs (**Gillespie and Flanders, 2010**).

Additionally, it is advised to formulate chicken diets to meet their energy requirements based on recommendations for particular strains, whereas increasing dietary energy levels to improve FCR results in an increase in fat deposition (**Fouad and El-Senousey, 2014**), especially in the early stages of broiler fattening (**Gous, 2010**).

Proteins

While providing energy has been the main focus of nutrition, chicks would benefit from a more balanced nutrient profile, especially protein and AAs. Protein is a crucial component of chicken diets, so when developing a broiler diet, the focus is mostly on the crude protein (**Beski, Swick and Iji, 2015**).

Because protein is one of the most expensive elements in chicken diets, meeting the protein needs of broilers accounts for a very significant portion of the cost of feeding (**Beski, Swick and Iji, 2015**). According to **Fouad and El-Senousey (2014)**, increasing the protein content of the feed in broilers increases average daily gain, carcass production, and carcass quality while decreasing body fat deposition, particularly the proportion of abdominal fat.

The value of a protein feed for chicken depends on its capacity to provide an adequate amount of the EAAs that chicken needs for muscle development, protein digestibility, vital functions, and disease prevention (**Beski, Swick and Iji, 2015**).

Broiler chicks tend to respond to AA shortages quickly by changing the amount and type of feed they consume (**Bunchasak, 2009**). Faster growing broiler strains need a higher AA to energy ratio because the AA requirements rise proportionately more quickly than the energy needs do (**Gous, 2010**).

The majority of an animal's protein needs are met by sources of plant protein, which are frequently less expensive than animal proteins. Maize and soybeans are the most popular sources of protein. However, other cereal grains like wheat and sorghum are also frequently utilized, in addition to some plant protein sources including canola, sunflower, and peas (**Beski, Swick and Iji, 2015**).

However, plant protein sources lack some essential amino acids (EAAs) and are nutritionally imbalanced, which reduces their biological value because they might not provide the limiting AAs necessary for chickens to produce eggs and meat. In order to form a balanced diet, plant proteins typically need to be supplemented with AAs or other sources of protein, including animal protein (**Akande et al., 2010**).

Contrarily, animal proteins are expensive for use in commercial broiler production while having a well-balanced composition of EAAs required for body growth and development. As a result, they are typically added to diets to balance out the AA levels rather than serving as the primary source of protein (**Denton et al., 2005**).

As was previously indicated, compared to the commercial broiler utilization in earlier years, broilers now attain their final weights in fewer days. As a result, the dietary AA requirements for modern broilers should be higher than those for broilers nowadays (**Dozier, Kidd and Corzo, 2008**). The addition of AA to chickens can change how their muscles develop and how their body proteins are made (**Nasr and Kheiri, 2012**).

Methionine, lysine, threonine, tryptophan, isoleucine, leucine, histidine, valine, phenylalanine, and arginine are the EAAs for poultry. Some people also believe that glycine is crucial for young birds. Because they may be produced from methionine and phenylalanine, respectively, cysteine and tyrosine are regarded as semi-essential AAs. Methionine, lysine, and threonine are the three EAAs that are most restricted in the majority of realistic poultry diets (**Matsushita, Takahashi and Akiba, 2007**), followed by lysine (**Siqueira et al., 2013**), and threonine (**Sá et al., 2007**).

The first EAA in the diet of chickens is generally acknowledged to be methionine, which reduces the biological value of protein (**Matsushita, Takahashi and Akiba, 2007**). Methionine is crucial for growth performance, lean meat production (**Fouad and El-Senousey, 2014**), improved poultry carcass quality (**Wu et al., 2012**), which is shown by a decrease in carcass fatness and an increase in carcass yield (**Bunchasak, 2009**), and immune and inflammatory responses (**Jankowski, Kubiska and Zduczyk, 2014**).

In fact, immune system cells need enough methionine in their diets to synthesize proteins (**Grimble, 2006**). Methionine enhances the immune response through both direct effects (protein synthesis and breakdown) and indirect effects involving methionine derivatives, claims **Bunchasak (2009)**. On the other side, methionine deficits that result from AA imbalances cause broilers to consume less feed.

So it stands to reason that methionine, by encouraging proper feed intake, minimizes losses and lowers production costs (**Bunchasak, 2009**).

According to **Siqueira et al. (2013)**, the capability for body protein deposition is strongly correlated with lysine, a limiting AA in poultry diets. By raising muscle pH, boosting protein synthesis, and decreasing water-holding capacity, it plays a significant influence on

meat quality. Lysine greatly improves the production of lean meat in poultry (**Tesseraud et al., 2009**).

Lysine has also been shown to play a role in the production of cytokines, lymphocyte proliferation, and the optimal performance of the immune system in chickens in response to infection (**Nasr and Kheiri, 2012**).

After methionine and lysine, threonine is the third most restricting amino acid in broiler diets. In comparison to methionine and lysine, broilers' threonine needs have received less research (**Sá et al., 2007**).

Threonine is used in a variety of fundamental metabolic activities in addition to protein synthesis. The precursor of glycine and serine, this AA takes role in the synthesis of feather protein. Being the primary AA in gamma serum immunoglobulin, it is also quite effective in immune response. Additionally, it contributes to the production of collagen and elastin and, when dietary crude protein is reduced, indirectly encourages a decrease in thermal stress (**Ospina-Rojas et al., 2014**).

Enzymes

To improve the digestibility of the various feed ingredients, enzymes are currently frequently added to chicken feeds (**Aviagen, 2009b**). Due to their capacity to control digestion and the different metabolic issues linked to high-fiber feed, carbohydrates (feed enzymes) have been used commercially in chicken nutrition since the late 1980s and early 1990s. According to **Aftab and Bedford (2018)**, enzymes are utilized to counteract the negative effects of non-starch polysaccharides on intestinal health and poultry production.

Minerals and vitamins

Mineral and vitamin supplements are frequently added to broiler diets within ranges intended to prevent deficiency or toxicity (**Waldenstedt, 2006**). Mineral and vitamin deficiencies in the diet can lead to metabolic problems that have a negative indirect impact on feed consumption. A bird may try to eat more food to meet its intake need if it has a little mineral deficiency. On the other hand, excessive dietary vitamins and minerals can be tasted by the bird and cause it to reject the feed (**Ferket and Gernat, 2006**).

The main role of vitamins and minerals in metabolism is to be as cofactors (**Ferket and Gernat, 2006**). Additionally, minerals are necessary for the development of the skeletal system,

general health, preservation of the body's acid-base balance, growth, immunological function, and FCR (**Aviagen, 2014a**).

Along with Na, K, Cl, S, Mg, Ca and P are considered macroelements since they are the most prevalent mineral elements in the body. Macroelements must be present in the diet in amounts more than 100 mg.kg⁻¹, according to **Ravindran (2013)**.

For the development and upkeep of the skeletal system as well as for optimal eggshell quality, Ca and P are essential (**Ravindran, 2013**).

Diets for broilers typically include additional Ca and P. The availability of Ca is often quite high from most sources; however, the availability of P varies significantly depending on the source. Because chickens lack endogenous phytase in their digestive enzymes, phytates, which make up a large portion of the P in cereal grains, are poorly available to them. The P requirement for poultry is given as non-phytate P rather than total P since it is generally accepted that roughly one-third of the phosphorus in plant feedstuffs is non-phytate and biologically accessible to broilers (**Waldenstedt, 2006; Ravindran, 2013**).

To maximize the absorption of these two minerals, the ratio of Ca to non-phytate P in the diets of growing birds must be kept at 2:1, since an aberrant ratio may be just as damaging as a shortage in either element (**Waldenstedt, 2006; Ravindran, 2013**).

The body's acid-base balance is controlled by the additional macroelements Na, K, and Cl in order to maintain the physiological pH. The metabolic processes are altered to maintain the pH if a movement towards acidic or basic circumstances takes place, which is likely to have a negative impact on performance. It's crucial that these three minerals are in general equilibrium. In hot areas, prevention of electrolyte imbalance requires careful thought. Their dietary levels must all fall within acceptable ranges, not be inadequate or excessive, for them to be effective (**CRF, 2009; Ravindran, 2013**).

Particularly in birds under heat stress (**Ravindran, 2013**), excessive levels of Na, P, and Cl might lead to increased water intake and associated litter quality difficulties (**Ferket and Gernat, 2006; Aviagen, 2014a**).

When it comes to microelements in the diet, they also serve a purpose for the body's systems as parts of larger molecules and as co-factors of enzymes in different metabolic operations. In contrast to macroelements, they are needed in very small levels, measured in parts per million (ppm) (**CRF, 2009**), and unless they are deficient for an extended period of time, they do not impact appetite.

The microelements Cu, I, Fe, Mn, Se, Zn, and Co are among them. The conventional cereal-based diets used for poultry need be supplemented with both macro and microelements. The biological availability of some microelements' organic forms is generally thought to be higher than that of their inorganic counterparts (**Ravindran, 2013**).

Vitamins are divided into water-soluble (vitamins B complex and C) and fat-soluble (A, D, E, and K) groups. Despite the fact that they are only slightly necessary, they are an essential component of a bird's diet and must be provided to prevent a deficiency, illness, or metabolic syndrome (**CRF, 2009**).

Since the bird can make its own vitamin C, it is not typically regarded as a dietary necessity. However, in difficult situations, like heat stress, vitamin C supplements may be helpful. Compared to other nutrients, vitamins have more intricate metabolic functions. Vitamins are players or mediators in all biochemical pathways in the body, not only simple body building components or energy sources (**Ravindran, 2013**).

Due to its antioxidative qualities, vitamin E or vitamin C can help reduce the tissue damage caused by lipid peroxidation in broilers under heat stress (**Whitehead and Keller, 2003**). Lipid peroxidation is the primary cause of tissue damage in broilers under heat stress.

According to **Leshchinsky and Klasing (2001)**, vitamin E is also necessary for the health and the operation of the immunological, muscular, circulatory, neurological, and reproductive systems.

However, when combined, vitamins E and A more nearly halved the malondialdehyde (a marker of lipid peroxidation) levels in the serum and liver of broilers under heat stress, but they had a much smaller impact when given alone (**Sahin et al., 2002**).

1.2.3 Characterization of Ross 308 hybrid combination

Few fast-growing commercial broiler strains are essential in supplying the world's population with the necessary quantity of chicken meat. With the progress of modern technologies applied in chicken nutrition as well as in genetics, the body weight gain of the broiler strains has been significantly boosted and the feed utilization has been much improved. Broiler strains are performing better than ever with to advancements in breeding and nutrition (**Hossain, Suvo and Islam, 2011**).

The broiler chicken hybrid Ross 308 is well-known around the world for consistently delivering excellent results. A three-linear, two-breed hybrid called Ross 308 was created by

Ross Breeders in the United Kingdom. Its ability for growth, feed effectiveness, strong performance, high carcass production, and uniform body muscles are valued by both cooperatives and independent farmers. At 42 days old, the broiler has a weight limit of 2652 g. Due to its qualities, this broiler can satisfy even the most discerning clients worldwide. When broiler performance is crucial, the Ross 308 breeder generates a large quantity of eggs with good hatchability to reduce chick costs (**Aviagen, 2014b**).

Achieving strong bird performance is necessary for the cost-effective production of chicken meat, and the following factors are crucial for the Ross 308 broiler to operate at its best:

- a) improving hatching, storage, and transport conditions to maximize chick quality;
- b) plan the brooding setup to provide for simple access to water and food upon placement and to make the switch from supplemental systems to the automated feeders and drinkers at 4 to 5 days as painless as possible;
- c) providing an excellent starting diet that is very digestible;
- d) monitoring the behavior of the chicks to keep them in their thermal comfort zone when the humidity is above 50%;
- e) starting a minimal ventilation program right away;
- f) keeping track of the crop fill, eating and drinking habits, and 7-day live weight to enable ongoing brooding setup improvement;
- g) preserving the birds' thermal comfort zones throughout the growing period; because fast-growing broilers generate a lot of heat, especially in the second half of the grow-out phase, maintaining ambient temperatures below 21 °C from the age of 21 days may promote faster growth rates;
- h) upholding the strict biosecurity and hygienic standards to minimize disease (**Aviagen, 2014b**).

1.3 Formation, composition and use of chicken fat

There is a great deal of concern regarding food fat intake in light of recent suggestions to lower the risk of cardiovascular disease (**Soriano-Santos, 2010**).

Saturated fatty acids (SFA), which have been linked to disorders connected with contemporary life, primarily in industrialized nations, **WHO (2003)** recommends that you consume no more than 10% of your total energy intake come from SFA. According to **Milićević**

et al. (2014), dietary intake of SFA and cholesterol is most closely linked to coronary heart disease and arteriosclerosis.

Since saturated fat intake is the primary dietary predictor of blood LDL cholesterol concentrations, **EFSA (2010)** recently decided not to propose a reference on cholesterol intake in addition to its conclusion on SFA intake and suggested continuation of the population recommendations regarding fat and SFA. Polyunsaturated fatty acids (PUFA) have been proposed as SFA's best alternative for lowering the risk of coronary heart disease. The ideal PUFA to SFA (P/S) ratio is over 0.4, with meat often having a P/S ratio of roughly 0.1 (**Milićević et al., 2014**).

Recent advances in the study of human food have generated a great deal of interest in n-3 and n-6 polyunsaturated fatty acids (PUFAs). When the first double bond is situated at the chain's third carbon from the methyl group (CH₃), a fatty acid is referred to as being -3, and when it is situated at the chain's sixth carbon from the same radical, it is referred to as being -6 (**Da Silva, Rocha and Quinto, 2015**).

Due to their roles in lowering the incidence of lifestyle diseases like coronary artery disease, hypertension, and diabetes (**Kalakuntla et al., 2017**), as well as their positive health effects like lowering triglyceride and cholesterol levels in blood, as well as their anti-inflammatory and anti-thrombotic activities (**Kaplan et al., 2016**), there is a growing interest in a balance of n-6 to n-3 FAs.

Despite the widespread suggestion, dietary n-3 long-chain PUFA intake has not dramatically increased over the past ten years. According to **Kalakuntla et al. (2017)**, adult daily intake of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) is much below the amount deemed necessary to avoid cardiovascular disease.

According to a number of studies, a dietary n-6/n-3 ratio imbalance can have an impact on one's health since it increases the production of pro-inflammatory cytokines, which is thought to be a risk factor for coronary heart disease and cancer. The optimal n-6/n-3 ratio, according to nutritionists, is thought to be around 4:1 (**Milićević et al., 2014**), but this ratio is currently significantly higher in Western diets (>10:1), which may be one of the main causes of many lifestyle problems (**Kalakuntla et al., 2017**).

As is generally known, as compared to other types of meat, chicken seems to have a low fat content. The amount of fat and cholesterol in a cut is mostly dependent on whether skin is present (**Bordoni and Danesi, 2017**).

According to **Pereira and Vicente (2017)**, when the skin is removed, chicken breast meat, which is about 1% leaner than other chicken cuts, is regarded to be the leanest meat. Additionally, compared to the low amounts of PUFA and high levels of SFA in red meats, the composition of poultry fat is advantageous nutritionally. However, both internal (age, gender, and genotype) and external (temperature, feeding) factors affect the FA profile of poultry meat (**Starčević et al., 2014**).

Only a third of total fat is made up of saturated fatty acids (SFA), and poultry flesh contains considerable levels of MUFA. Long chain n-3 PUFA, such as α -linolenic acid (ALA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3), are another important dietary source found in poultry meat. Thus, poultry meat may serve as a significant source of n-3 FAs in the majority of Western nations where fish consumption is generally low (**Marangoni et al., 2015**).

n-6 FAs, particularly linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6) are more prevalent in skin than other types of meat (**Marangoni et al., 2015**). Table 3 compares the pertinent n-6 and n-3 PUFA contents.

Table 3: Content of relevant n-6 and n-3 PUFAs in selected meats and egg (**Marangoni et al., 2015**)

Parameter	18:2 n-6	20:4 n-6	18:3 n-3	20:5 n-3
Poultry meat	1,443	98	73	5
Chicken (with skin)	2,880	80	140	10
Chicken (without skin)	550	80	20	10
Turkey (with skin)	1,700	110	110	0
Turkey (without skin)	640	120	20	0
Pork	831	68	53	3
Beef rib eye	240	20	10	NA
Beef sirloin	94	9	20	5
Goat and mutton	460	64	178	5
Lamb	369	84	54	5
Egg	1,272	156	31	0

Note: Values are presented in mg per 100 g of edible portion. PUFA = monounsaturated fatty acids; 18:2 cis n-6 = linoleic acid; 20:4 = arachidonic acid; 18:3 n-3 = α -linolenic acid; 20:5 n-3 = eicosapentaenoic acid; NA = not available.

Through nutritional manipulation, saturated fats are increasingly being replaced by unsaturated fats in poultry products due to the known health benefits of PUFA on human health **(Ahmed et al., 2015)**. One of the most effective strategies to increase the accumulation of desired PUFA in chicken meat and so provide this nutrient to humans is to modify the composition of the diet's FAs **(Kalakuntla et al., 2017)**.

According to the Organization for Economic Cooperation and Development (OECD) and the Food and Agricultural Organization (FAO), the worldwide per capita consumption of chicken meat in the last decade has increased by 15%, growth which has outstripped that registered for beef and pork. The main consumers are the United States and Brazil, whose annual consumption exceeds 40 kg per capita **(FIRA, 2019)**. Such an increase in the consumption of chicken meat is mainly due to the perception by health-conscious consumers that chicken meat is a low-fat source of healthy nutrition, rich in unsaturated fat and a high in protein **(Mozdziak, 2014)**. In addition, chicken meat is increasingly used in the development of new chicken-based convenience products (chicken bologna, chicken nuggets, chicken hot dogs, chicken wings), which have been successfully marketed for consumption at home and also in the growing fast-food industry **(Choi et al., 2016)**.

However, the rapid growth of poultry production has led to the massive generation of food-processing by-products like bones, viscera, abdominal fat, feet, head, blood and feathers. If these by-products were regarded as having greater nutritional value, their use would contribute to the development of a sustainable food industry while increasing the value of this sector **(Herrera, 2008)**.

Until now, these by-products have only been sold as animal feed and to pet food processors **(El Boushy and Van der Poel, 1994; Barbut, 2015; Vikman et al., 2017)** and, recently, for the production of biodiesel **(Abid and Touzani, 2017)**. However, there are no references about the possible use of some of these by-products as raw materials for use in human food processing. For example, it may be possible that the abdominal and gizzard fat that remains inside the poultry carcass, where it represents approximately 2–2.5% of the total weight of the slaughtered chicken **(Chiu, Gioielli and Sotero-Solis, 2002)**, could be used as fat source for the production of chicken sausages or other meat products, especially taking into account its characteristic content of unsaturated fatty acids. Until now, this abdominal and gizzard fat has been discarded by small producers, together with the viscera, feathers and blood, thus creating an environmental problem. Formation of abdominal and gizzard fat as the main chicken fat

by-products is shown in Figure 1 (Peña-Saldarriaga, Fernández-López and Pérez-Álvarez, 2020).

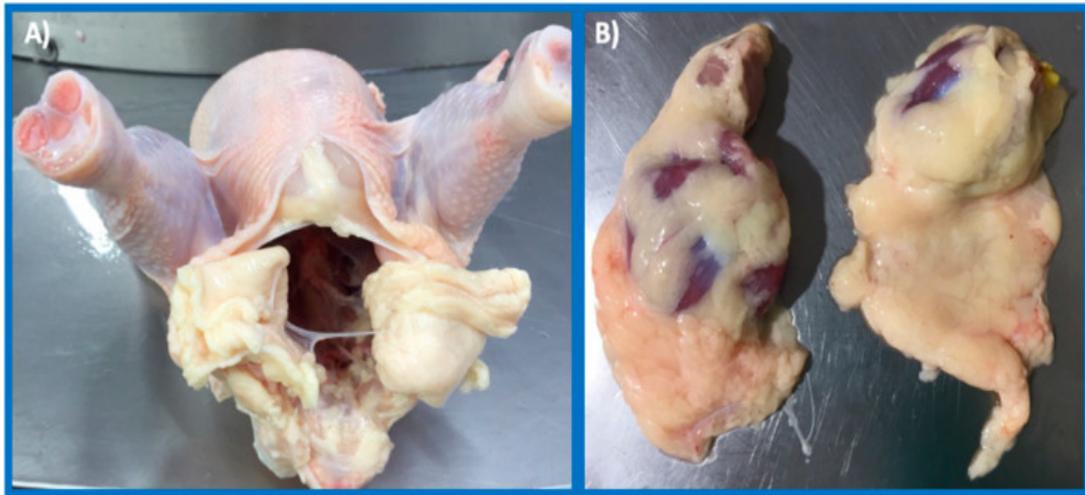


Figure 1: Formation of abdominal (a) and gizzard (b) fat in the chicken carcass (Peña-Saldarriaga, Fernández-López and Pérez-Álvarez, 2020)

As explained above, the fat deposits of a chicken carcass come mainly from the diet, so that the lipid profile in these tissues reflect the lipid profile of the diet (Sirri et al., 2003). The interactions that take place between the nutrients that compose the diet and the synthesis and activity of lipogenic enzymes are responsible for a wide range of possibilities regarding lipid deposition in adipose tissue. Moreover, the biological activity of some fatty acids stimulates or inhibits specific lipogenic genes encoding enzymes (Jump, 2002).

The fact that the lipid profile of chicken fat by-products from the 3 farms under study of Peña-Saldarriaga, Fernández-López and Pérez-Álvarez (2020) did not show differences is probably due to the stability of the feed used in each farm. Since the feeding and other farms' routines were the same in all three farms, so that the only difference was their respective geographical location and climatic conditions, it seem safe to conclude that neither factor was important enough to modify this composition. This is very important because if chicken fat by-products are to be used as fatty ingredients in the meat industry, the greater the homogeneity in their composition, the easier it will be to formulate meat products.

Of other sources of animal fat commonly used in meat products, chicken fat by-products have the highest amount of unsaturated fatty acids (UFA, 65.5%) and bovine tallow the lowest

(44 – 50%). It must be noted that the chicken fat by-products analysed in this study contained a higher proportion of polyunsaturated fatty acids (PUFA, approx. 40% of total UFA) than pork or beef fat (has less than 20%). Unsaturated fatty acids include essential fatty acids that play beneficial roles in human health. Oleic acid may help decrease the circulating concentration of low density lipoprotein (LDL) cholesterol in humans and is considered a “healthy” fat (**Kwon and Choi, 2015**). High oleic acid values are desirable for their hypocholesterolemic action, and have the added advantage of not lowering high density lipoprotein (HDL) cholesterol (“good cholesterol”), and protecting against coronary heart diseases (**Ospina et al., 2010; Da Silva et al., 2018**). The essential fatty acids include the w3 and w6 families, which are not biologically synthesized by humans, but which are necessary for biological processes and therefore should be included in the human diet (**Da Silva et al., 2018**).

By contrast, the highest saturated fatty acid (SFA) levels are found in beef tallow (46 – 55%) and the lowest in poultry fat by-products (30.2%). Taking into consideration that the high consumption of saturated fatty acids has been associated with increased levels of serum cholesterol and LDL, both risk factors for cardiovascular diseases (**FAO, 2010; Liu et al., 2017**), using chicken fat by-products as fatty raw material in the meat industry could be considered advantageous. However, some studies suggest that the role of saturated fat in heart diseases is complex because of the heterogeneous biological effects of different saturated fatty acids and the diversity of food sources (**Mozaffarian et al., 2010; Liu et al., 2017**), so that not all SFAs should be considered hypercholesterolemic. These findings suggest that the specific matrix of different foods, including other fatty acids, nutrients, and bioactives, may biologically modify the effect of saturated fat in cardiovascular diseases.

According to (**French, Sundram and Clandinin, 2002**) the most undesirable fatty acid is myristic acid, which only represents 1.3% in chicken fat by-products (Table 3), 3% in beef tallow and 3.5% in pork back fat (Table 5). Several authors have reported that palmitic acid has a low hypercholesterolemic effect and stearic acid has no effect because it becomes oleic acid in the body (**Sinclair, 1993**) and so does not influence blood cholesterol levels.

These results suggest that chicken fat can be used as fatty ingredient in formulating sausages, for example, as a partial or total substitute of traditional solid fat sources with their higher SFA concentrations, or be used together with chicken skin, thus increasing the amount of useful fat that can be obtained from poultry (**Peña-Saldarriaga, Fernández-López and Pérez-Álvarez, 2020**). In addition, the high levels of UFA in chicken fat by-products could

allow them to be used as frying oil as well as mixed with other solid fats to increase their plasticity.

1.3.1 Adjusting of chicken fat composition

Fats are more variable compared to proteins, the average fat content in breast muscle is lower (1.3%) compared to thigh muscle (4.5%) in conventionally raised chickens. Turkey meat and meat from non-conventionally raised chickens are slightly leaner (0.8% in breast muscle), while duck meat is fattier (more than 2% in breast muscle according to the hybrid). Within lipids, the most variable fraction is triglycerides, the amount of which is positively correlated with the total lipid content: 0.7% in breast and 3% in thigh muscles of chickens and 0.5 – 0.8% in ducks. The content of phospholipids is 0.6% in the breast and 0.8% in the thigh muscle of chickens and 1.1% in ducks, and the cholesterol content is 0.05% in the breast muscle and 0.09% in the thigh muscle of chickens and 0.07 and 0.12% in ducks (**Rabot, 1998; Baéza, 2000**).

The feed affects the content of intramuscular fat mainly by its energy value and energy:protein ratio, or when the intake of essential amino acids (lysine, methionine) is lower than necessary for proper growth. By increasing the content of lipids in the feed by 30 or 90 g.kg⁻¹, their content in the breast muscle of turkeys increased by 14, respectively 40% and in the thigh by 27, respectively 53% in parallel with the increase in body weight compared to the control group (**Salmon and Stevens, 1989**).

In the same study, when the energy:protein ratio was increased from 65 to 83 KJ.g⁻¹, lipid content in breast muscle decreased by 27% and in thigh by 5%, concomitant with a reduction in body weight. **Conde-Aguilera et al. (2013)** compared two levels of methionine intake

in the diet of chickens between the 7th and 42nd day. By reducing this intake by 34% compared to the control group, fat content increased by 28% at 42 days of age.

In contrast, in a study (**Baéza et al., 2015b**), the energy source in the diet (carbohydrates or fats) had no effect on the intramuscular fat content of chicken meat.

Dietary restriction generally reduces intramuscular fat content, while overfeeding, practiced in ducks and geese, doubles this content (**Baéza, 2000**).

The lipid content in poultry meat is also influenced by age, hybrid and production system. For example, in heavy hybrids of conventional chickens, breast muscle lipid content

increased from 1.29 to 1.68% between 35 and 63 days of age (**Baéza et al., 2012**), and in ducks, breast muscle lipid content increased from 1.79 to 2.74% between 8 and 13 weeks of age (**Baéza et al., 2000**).

By comparing 5 chicken hybrids with different growth rates and therefore different slaughter ages and slaughter weights of 1.5 – 2 kg, **Tang et al. (2009)** demonstrated that the average lipid content in breast and thigh muscles ranged from 0.96 to 1.42%. Older chicken hybrids with very slow growth are generally fatter than commercial hybrids because they have not been selected for rapid fattening.

The FA in chicken consists of approximately one-third saturated fatty acids, one-third monounsaturated FA, and one-third polyunsaturated FA (**Rabot, 1998**).

Oleic acid is the main monounsaturated FA, followed by palmitoleic acid. The main polyunsaturated fatty acids are linoleic acid and arachidonic acid. Total lipids in chicken also contain linolenic acid and long-chain n-6 and n-3 polyunsaturated fatty acids. The main factor of variation in the composition of FA from poultry meat is the composition of FA in the feed. It appears that feeds with a higher content of saturated FA generally increase their content in chicken meat, especially the content of palmitic and stearic acids. On the other hand, vegetable oils with a higher proportion of unsaturated FA in feed also increase their content in meat (**Lessire, 2001**).

Since 2001, fats of animal origin (tallow and lard) have been replaced by vegetable oils (rapeseed, soybean or linseed) in poultry feed, which has increased the proportion of polyunsaturated fatty acids in poultry meat. Currently, thanks to a more varied diet, conventional chicken hybrids have a high content of polyunsaturated FA in the meat (30% of the total FA in the breast muscle) and also a higher content of fat in the meat (1.25%) (**Chartrin et al., 2005**).

In Western countries, the daily intake of FA is not satisfactory because the n-6 FA:n-3 FA ratio is around 15, with a recommended value of 5. Several studies have been conducted to enrich fresh chicken meat with n-3 essential acids. The most effective way is to use fish oils rich in n-3 long-chain polyunsaturated FA. However, fish oils left an undesirable smell in the meat, and their suitable alternative is, for example, replacement with microalgae. It is also possible to use linseed or rapeseed oils, which are rich in linolenic acid, although in this case the proportion of n-3 long-chain polyunsaturated FA stored in the muscles remains low. Combining extruded flaxseed with microalgae in conventional chicken feed has been shown to enrich meat with linolenic acid and long-chain n-3 FA with an n-6 FA/n-3 FA ratio of 3.65

compared to a soybean oil-based feed rich in n-6 FA and a n-6 FA/n-3 FA ratio of 11.52 (Baéza et al., 2015a).

Marcinčák et al. (2018) state that feeding fermented feeds can improve the fatty acid profile of chicken meat. The authors report that feeding 10% maize meal fermented with *Umbelopsis isabellina* CCF2412 resulted in an increased proportion of gamma-linolenic, alpha-linolenic and oleic acids in breast muscle fat and an improved ratio of n-6 to n-3 polyunsaturated FA in fresh meat. These authors also documented that fermented feed improved the quality, oxidative stability and sensory properties of broiler chicken meat.

Contrary to this study, Chung and Choi (2016) reported that feeding koji (*Aspergillus oryzae*) with 1% fermented red ginseng pomace did not affect the fatty acid profile of breast and thigh muscle of broiler chickens.

Several studies have also investigated the fortification of poultry meat with conjugated linoleic acid (CLA). Du and Ahn (2002) and Sirri et al. (2003) tested different levels of dietary CLA intake in chickens from 0.25 to 4% for 3 to 5 weeks. CLA deposition in meat increased with increasing CLA content in the feed, while oleic, palmitoleic and arachidonic acid contents decreased.

Combined dietary intake of CLA with fish oil increased the storage efficiency of n-3 long-chain polyunsaturated FA and CLA in breast muscle. Alternatives to soybean oil as a source of fat in feed can also be fat from insect larvae. As a result of its use, the proportion of saturated micronutrients (especially lauric and myristic acids) in chicken meat (breasts and thighs) increased at the expense of polyunsaturated micronutrients. The n-6 FA : n-3 FA ratio also increased (Schiavone et al., 2017, Cullere et al., 2019).

The feed energy source can also affect the composition of FA in poultry meat. A feed mixture with a high carbohydrate content promotes liver lipogenesis and thus the synthesis of saturated and monounsaturated FA, while a feed with a high fat content is more likely to promote the direct deposition of FA in peripheral tissues (Baéza et al., 2015b).

In an extreme case of overfeeding, the daily intake of carbohydrates (corn starch) is very high and liver lipogenesis is strongly stimulated, especially the synthesis of palmitoleic and oleic acids, which are then stored in peripheral tissues (fat and muscle tissues). In the breast muscle of overfed ducks, the proportions of monounsaturated and polyunsaturated FA are 50 and 16% of the total FA, compared to 36 and 31% in the breast muscle of traditionally fed ducks (Girard et al., 1993).

Depending on the studies, the FA composition of poultry meat may or may not change during storage, either at refrigerated or frozen temperatures. Heat treatment may or may not have an effect on the FA composition. For example, **Baéza et al. (2013)** demonstrated that the process of canning and cooking had little effect on the composition of FA in breast fillets.

The same was true after 30 minutes of heat treatment of the ground turkey mixture at 80 °C (25% breast, 25% thigh, 50% mechanically separated meat; **Ahn et al., 1993**).

The content of CLA in chicken thighs also changes little during baking, but its content decreases when the meat is boiled or fried (**Franczyk-Zarow et al., 2017**). When cooking a whole chicken, the overall composition of the meat changes, as fatty acids from the subcutaneous fat tissue migrate into the muscle tissue (**Rabot, 1998**).

1.4 Antibiotic resistance in poultry industry

Diseases in the production of chicken have long been treated using antibiotics (ATBs). However, the original purpose of using ATB, which was a therapeutic effect, has been expanded to other goals like improving animal welfare, increasing economic efficiency of rearing, and prophylactically treating diseases that are primarily brought on by stressful situations during transport (**Allen et al., 2013**).

The significant increase in the growth rate of broiler chickens over time is presumably the result of antibiotic growth promoters (AGPs), which have been added to poultry feeds (**Millet and Maertens, 2011**). Any medication that kills or inhibits bacteria and is given at a low, subtherapeutic dose is referred to as a "antibiotic growth promoter" (**Hughes and Heritage, 2004**).

The development of livestock production has given rise to the usage of ATBs for growth enhancement. The use of subtherapeutic ATBs and antimicrobial drugs to combat infectious pathogens has been demonstrated to be effective in reducing the productivity of farmed food animals. Due to the discovery of bacteria with ATB resistance in animals fed the AGPs, there have been concerns about the development of resistant bacteria that are pathogenic for both animals and humans (**Allen et al., 2013**). As a result, the use of in-feed ATBs is largely a problem of intensive farming methods (**Hughes and Heritage, 2004**).

ATB resistance has expanded globally as a result of the unnecessary use of antimicrobials in human and veterinary treatment (**Robert et al., 2015**). Particularly when the same classes of antimicrobials are being used in humans, wealthy countries are more likely than

developing ones to experience major public health issues as a result of their frequent usage **(Hughes and Heritage, 2004)**.

According to **Peng, Salaheen and Biswas (2014)**, "antibiotic resistance" refers to a microorganism's capacity to live, develop, or reproduce despite the presence of ATB. The possibility that people could contract the sickness caused by these bacteria in animals raises more questions about the evolution of ATB resistance **(Pugh, 2002)**.

Recent research has demonstrated that ATB use that is only temporary and unneeded can stabilize resistant bacterial strains in the human intestine for many years. Due to the high intensity and duration of the therapy in this situation, it is not only much more difficult but also more expensive **(Sharma et al., 2014)**.

The plasmid DNA contains resistance genes that can be traded between bacteria. ATB resistance can therefore spread through bacterial populations when new generations inherit ATB resistance genes as well as when bacterial species share or exchange genetic material with one another **(Stanton, 2013)**.

Between harmful bacteria and the gut microflora, the transfer of resistance genes frequently takes place. The commensal microflora of animals may select resistant bacteria, increasing their numbers in the intestine and in the feces as a result of the AGPs used in animal husbandry. According to **Nemcová et al. (2010)**, the reservoir of resistance genes facilitates the transfer of resistance genes to harmful bacteria in humans. Consuming animal products with ATB still present, such as muscle and liver tissue, can also be risky **(Robert et al., 2015)**.

The European Commission voted to outlaw (1 January 2006) the use of ATBs as growth promoters in feed throughout the European Union due to the rise of microbes resistant to ATBs, which are used to treat human and animal illnesses. Nowadays, antimicrobial drugs should only be applied to treat animal infections, and this application should be supported by evidence that has been verified by science. However, despite this, Australia and North America both continue to use antimicrobial drugs for food animals inappropriately **(Hammerum et al., 2007)**.

1.5 Feed supplements in poultry nutrition

Poultry industry constitutes one of the most significant segments of the agricultural and veterinary sector worldwide. For this reason, one of its main objectives is to improve not only the quantity but also the quality of the offered product. Over the years, the abuse of antibiotics as growth promoters has led to the development of antibiotic-resistant bacterial strains,

imposing the need to find alternative solutions for animal welfare and for gastrointestinal diseases prevention, finding confirmation in the use of pre and probiotics. Various stressors, together with feed deprivation during the first few hours of chicks' life increase the chance of disease contraction and mortality. Innovative feeding systems to be administered to chicks immediately after hatching in incubation rooms are gaining ground in poultry industry. These new systems consist of complementary feeds, which provide all the nutrients and additives that chicks need during their first hours of life, promoting the development of the gastrointestinal system and preventing the documented side effects caused by fasting (**Riva and Monjo, 2020**).

As with other industries, its main purpose is to produce the maximum with minimal input. The constant increase of ingredients' price for chickens feeding, and the consequent farmers' lower profit, are highlighting the need for a balanced and effective feed, being it the most important requirement for an economic poultry production. In recent years, many feed additives, such as antibiotics, have been extensively used to promote the rapid growth of the animals. However, this abuse led to the development of bacterial strains resistant to antibiotics, with dangerous residual effects also in eggs and meat, being a potential risk for consumers' health. Therefore, the possibility of having to cease the use of antibiotics for disease prevention and as growth promoters, considering all the harmful secondary effects, has created the need both in the consumer and in the manufacturer, to seek new alternatives (**Trafalska and Grzybowska, 2004; Nava et al., 2005**).

1.5.1 Feed probiotics

Since dietary usage of probiotics has been linked to favorable effects on animal health and growth, they are seen to be a suitable replacement for subtherapeutic ATBs in poultry.

The term "probiotic" was initially used by **Lilly and Stillwell (1965)** to refer to "growth promoting factors" made by microorganisms. The words "probiotic" and "bios," which both mean "for life," are whence the term probiotic derives its etymology (**Moghaddam, 2011**).

The concept of probiotics has changed over time in tandem with the rise in popularity of using supplements containing live bacteria and in light of advancements in our knowledge of their mechanisms of action (**Kechagia et al., 2013**). The term "probiotic" has been given many definitions. According to **FAO/WHO (2002)**, the most frequently recognized definition is "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." According to this criteria, a probiotic's ability to improve the balance of the GI tract must be proven (**Giannenas et al., 2012; Daneshmand et al., 2015**).

According to **Parvez et al. (2006)**, the following capabilities are required for microorganisms to be employed as probiotics:

- a) have a positive impact on the host;
- b) survive incorporation into food at high cell densities and continue to exist throughout the product's shelf life;
- c) endure passage through the GI tract;
- d) cling to and colonize the lumen of the tract's intestinal epithelium;
- e) create pathogen-targeted antibacterial compounds;
- f) maintain gut microbiota stability and be linked to health advantages.

Probiotics are widely known to have a variety of varied effects on the host. Different species may have an impact on the mucosal immune system, epithelium and mucosal barrier function, and gut luminal environment. The genera and species of probiotic bacteria differ significantly from one another. These variations could be the result of different probiotics' mechanisms of action (**Nagpal et al., 2012**).

Probiotics exhibit numerous mechanisms of action, according to **Fallah, Kiani and Azarfar (2013)**:

- Pathogen-fighting activity by secreting substances such as bacteriocins, organic acids, and hydrogen peroxide that prevent the growth of the bacteria;
- Competitive exclusion, which involves competition for spots to cling to the mucous membranes of the intestine and prevents pathogenic germs from residing in the digestive system;
- The struggle for nutritive resources.

Probiotics are a group of species of bacteria, yeast, and mold. The study by **Amara and Shibl (2015)** mentions the species of each that are most prevalent:

- 1) *Lactobacillus: acidophilus, sporogenes, plantarum, rhamnosus, delbrueckii, reuteri, fermentum, lactis, cellobiosus, brevis, casei, farciminis, paracasei, gasseri, crispatus*;
- 2) *Bifidobacterium: bifidum, infantis, adolescentis, longum, thermophilum, breve, lactis, animalis*;
- 3) *Streptococcus: lactis, cremoris, salivarius, intermedius, thermophilus, diacetylactis*;

- 4) *Leuconostoc mesenteroides*;
 - 5) *Pediococcus*;
 - 6) *Propionibacterium*;
 - 7) *Bacillus*;
 - 8) *Enterococcus faecium*;
- Yeasts and molds: *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Aspergillus niger*, *Aspergillus oryzae*, *Candida pintolopesii*.

Due to the synergic adhesion effect, probiotic strain combinations may have greater health advantages than single strains (**Musa et al., 2009**). Lactic acid bacteria, particularly *Lactobacillus* spp. and *Bifidobacterium* spp., are the most well-known and often utilized probiotics (**Parvez et al., 2006**).

Probiotic administrations through water or feed have been used extensively. Although the details of selection for the poultry probiotics were frequently rarely reported, probiotic supplementation in farm industries dates back to the 1960s (**Park et al., 2016**). Probiotics can improve intestinal health by regulating the microbiota, stimulate the immune system's growth and function, increase nutrient synthesis and bioavailability, and lower the risk of certain diseases (**Parvez et al., 2006; Hemarajata and Versalovic, 2013; Amara and Shibl, 2015**).

There have been many research on the immunomodulatory effect. There is some uncertainty regarding the precise mechanisms through which probiotics exert their immunomodulatory effects. According to **Hemarajata and Versalovic (2013)**, probiotics influence the intestinal immune system by secreting substances and metabolites that have an impact on the development and operation of intestinal immune and epithelial cells.

Epithelial cells, dendritic cells, monocytes/macrophages, B cells, and T cells are among the several cell types impacted by probiotics. Probiotics also induce phagocytosis and IgA secretion, enhance Th1 responses, and attenuate Th2 responses, which all have been shown to have an impact on several components of the acquired and innate immune response (**Nagpal et al., 2012; Kechagia et al., 2013**).

It has also been demonstrated that probiotics, particularly lactobacilli, can modify the systemic antibody response to antigens in chickens (**Huang et al., 2004**). Both layers and meat-type hens' immune systems were modulated by the lactobacillus strains that were examined in the study by **Koenen et al. (2004)**.

As previously mentioned, probiotics in poultry diets not only boost immune function but also improve feed intake and digestion, maintain healthy intestinal microflora through competitive exclusion and antagonistic interactions, and change metabolism by boosting digestive and decreasing bacterial enzyme activity and ammonia production (**Kabir, 2009**).

Various methodologies and techniques, such as targeted, culture-dependent methods and metagenomic sequencing, have been used to examine the impact of probiotics on the composition, diversity, and function of the gut microbiota (**Hemarajata and Versalovic, 2013**).

Beneficial and unfavorable bacteria are in equilibrium in the GI tract of healthy, unstressed broilers. The broiler performs at its peak growth efficiency when there is a balance; however, when stress is applied, the beneficial microbiota tends to decline, which may lead to a high vulnerability to diseases (**Blajman et al., 2015**).

Ingested and temporary harmful microbes are prevented from adapting by a healthy balance of helpful microorganisms that are supplied by probiotic bacteria. To have the optimum outcomes in poultry, probiotic supplements must be applied as soon as feasible (**Edens, 2003**).

A chick must start creating a protective gut microflora as soon as it hatches into a setting that is highly polluted by bacteria, viruses, and protozoans. At the moment of hatching, the chick's GI tract is practically devoid of helpful bacteria, and in some cases, it takes five to seven days to build a healthy population of probiotics in the gut (**Edens, 2003; O'Dea et al., 2006**).

The formation of a healthy GI microbiota in newly hatched broiler chicks offers essential protection against these unfavorable organisms since it is during this early stage, when a stable gut microflora has not yet been established, that the chick is most prone to colonization by pathogens. Probiotics are believed to help the growth of a balanced GI microbiota (**Edens, 2003; O'Dea et al., 2006**).

Probiotics may also show to be an effective way to enhance broiler wellbeing because they are frequently exposed to a variety of environmental challenges. Broilers encounter stressful situations during high stocking densities, transportation, processing at the hatchery, and their adaption to the post-hatching phase (**Park et al., 2016**).

According to a study by **Pivnick and Nurmi (1982)**, the GI microbiota can stop the colonization of harmful microbes. The scientists looked into how hens' gut microbiota protected them from Salmonella infection during the first week after hatching. They discovered that giving the chicks intestinal microbiota that had been isolated from the GI tract of healthy adult chickens may protect them from infection. Salmonella adult-type resistance was attained by

oral feeding of this ill-defined mixed intestinal tract culture. The Nurmi notion or competitive exclusion is the name given to this practice afterwards (**Schneitz, 2005**).

Furthermore, it was shown that the amounts of nitrogen compounds that could harm the small intestine's epithelium were significantly reduced after probiotics had been applied to the GI tract. The optimal functioning of the GI tract may be positively impacted by the low concentration of nitrogen molecules and the larger concentration of free FAs. The optimization has a growth-stimulating impact and causes animals to gain more weight (**Nemcová et al., 2010**).

Probiotics have been the subject of numerous research, and the results show that they have a good impact on a number of chicken organism parameters. In addition to their effects on intestinal balance (**Shim et al., 2012**), morphological changes of the intestinal wall (**Islam et al., 2014**), immune response, and bone strength of broiler chickens (**Khaksefidi and Rahimi, 2005**), probiotics have also been shown to improve the performance of birds and the composition of their carcasses (**Sarangi et al., 2016**).

Additionally, adding probiotics to feed has shown promise as a natural strategy to enhance the quality of poultry meat. This is why more and more probiotic products are being created and used in poultry feeding (**Popova, 2017**).

It is interesting that prebiotics can improve the effects of administered probiotics and the good bacteria in the GI tract (**Bajaj, Claes and Lebeer, 2015**).

Prebiotics are indigestible food components that specifically promote the development and activity of gut microbial populations (**Hernández and Gil, 2016**). As 'food' for the probiotic bacteria, prebiotics function similarly to probiotics. Symbiotic refers to the relationship between a probiotic and a single prebiotic; the prebiotics are complimentary, and the probiotics are synergistic, resulting in a multiplier effect on each of their unique functions (**Flesch, Poziomyck and Damin, 2014**). Pre- and probiotics combined in one product have been found to have advantages over each taken separately (**Sarangi et al., 2016**).

Due to their various mechanisms of action, many feed supplements now in use do not strictly fall within the prebiotic classification, but they can nevertheless contribute to a healthy GI tract microbiome. According to **Kogut and Arsenault (2016)**, these compounds can be referred to as prebiotic-like chemicals.

Due to their prebiotic contents, bee products like bee pollen and propolis are also becoming acknowledged as compounds that can encourage the growth and/or activity of probiotic microbes (**Mohan et al., 2017**). **Guldás (2016)** looked into the effects of bee pollen

as a growth factor on a variety of probiotic bacteria and found that it had a favorable impact on both lactic acid generation and bacterial growth.

Overall, there is still a dearth of scientific studies investigating the possible benefits of probiotics and bee products in animals; hence, more study on this subject is urgently needed.

1.5.2 Bee pollen

Bee pollen (BP) is a mixture of flower pollen that honey bees (*Apis mellifera* L.) collect from plant anthers, mix with a little amount of salivary gland secretion or nectar, and store in special baskets called corbiculae that are located on the tibia of their hind legs. They are referred to as pollen burdens. According to several studies (**Oliveira et al., 2013, Attia et al., 2014, Komosinska-Vassev et al., 2015**), field bees gather and carry pollen to the hive.

The pollen basket that is brought to the hive typically contains material from a single plant, although occasionally bees will gather material from a variety of different plant species (**Dubtsova, 2009**).

Following the addition of various enzymes, nectar or honey, and wax, the bees insert the pollen in honeycomb cells with their legs as it undergoes anaerobic fermentation and is conserved thanks to the emerging lactic acid. Beekeepers refer to this pollen reserve as "bee bread" (**LeBlanc et al., 2009**).

Bee bread serves as the primary source of protein for bees and satisfies their nutritional needs for lipids, minerals, and vitamins (**Avni et al., 2014**). Additionally, worker bees use it as a source of nutrients and minerals for the royal jelly they manufacture (**Komosinska-Vassev et al., 2015**).

In general, it has been difficult to quantify the nutritional differences between pollen levels that have been collected and stored in hives. Regarding the nutritional qualities associated to the stored pollen, there aren't many research in the literature (**Kaplan et al., 2016**).

Although the chemical makeup of BP is extremely complex and nutritional, its constituents vary within a minimum and maximum range of values, primarily because of the source plant's geographic location and agroclimatic conditions, soil type, beekeeper activities, and storage (**Morais et al., 2011; Araújo et al., 2017**).

Depending on the plant type, the color of BP can range from brilliant yellow to black (**Dubtsova, 2009**).

There are around 250 different compounds in BP. According to **Pascoal et al. (2014a)**, the main components of BP are crude fibers, proteins, carbs, and lipids in amounts ranging from 13 to 55%, 0.3 to 20%, 10 to 40%, and 1 to 10%, respectively. Minerals and trace elements, vitamins (B complex, C, D, and E), carotenoids, phenolic substances, flavonoids, sterols, and terpenes are other minor components (**Mohdaly et al., 2015**).

Table 4: Average chemical composition (some basic components) of bee pollen (**Sagona et al., 2017**)

Parameter	Amount (%)
Dry matter	84.90
Total ash	2.47
Protein	25.30
Lipids	1.29
Carbohydrates	56.10

It is important to note that BP loses some of its bioactivity over time, and that conditioning fresh pollen before storage affects the nutritive and functional value of the substance (**Conte et al., 2017**).

According to **Ares et al. (2018)**, BP contains a variety of carbohydrates, including reducing sugars, polysaccharides, starch, and soluble and insoluble fibers. According to **Qian et al. (2008)**, the precise type of pollen's carbohydrate content has not yet been determined.

An average of 30.8% of the pollen contains digestible carbohydrates, the majority of which come from the nectar that the pollen from the flower has been mixed with. About 25.7% of this product is made up of reducing sugars, namely fructose and glucose (**Komosinska-Vassev et al., 2015**).

In spite of geographical disparities, a study by **Qian et al. (2008)** found that the levels of fructose, glucose, and sucrose—which make up around 90% of all low molecular weight sugars—are very similar. Another study (**Silva et al., 2006**) has shown that BP from the stingless bee *Melipona subnitida* has a significant amount of mannitol (34.9% dry weight).

Enzymes and both required and non-essential AAs are examples of proteins, which are among the primary elements of BP (**Ares et al., 2018**). The most significant source of protein and free AAs for bees is unquestionably plant pollen. When compared to plant pollen,

bee-collected pollen has a significantly higher protein content, albeit the amount varies substantially depending on the plant source (Da Silva et al., 2014b).

Pollen's protein concentration is seen as a clear indicator of how nutrient-dense it is. However, as the nutritional value is diminished as soon as insufficient amounts of the EAAs are present, the AA composition may more properly characterize the pollen's nutritional value than protein content (Human and Nicolson, 2006). According to Morais et al. (2011), BP is described to as "the only perfectly complete food" because it contains all the EAAs needed by the human body.

In pollen collected by honeybees, protein contents have been found to range from 2.5 to 61% (Avni et al., 2014). According to Komosinska-Vassev et al. (2015), BP typically comprises 22.7% protein, including 10.4% EAAs.

Table 5: Fatty acid composition of bee pollen (Sagona et al., 2017)

Fatty acid	Amount (% of total FAs)
C18:1 cis	25.20
C18:3 n-3	14.70
C18:2 trans n-6	12.40
C17:0	11.10
C16:0	10.50
SFA	36.40
MUFA	25.60
n-6 PUFA	18.50
n-3 PUFA	14.70
n-6/n-3	0.17

Note: Data are averaged from five bee pollen samples. C18:1 cis = oleic acid; C18:3 n-3 = α -linolenic acid; C18:2 trans n-6 = linoleic acid; C17:0 = heptadecanoic acid; C16:0 = palmitic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

EAA concentrations in BP ranged from 34.59 to 48.49% of the total AAs, according to Nicolson and Human (2013). Similar to this, Szczêsna and Rybak-Chmielewska (2000) found that roughly 37.5% of the overall AA content in BP is made up of EAAs.

According to **Sattler et al. (2015)**, the AA profile of BP is a possible tool for botanical or even geographic differentiation. The most prevalent amino acids (AAs) in pollen protein have been identified as glutamic acid, aspartic acid, proline, leucine, and lysine (**Taha, Al-Kahtani and Taha, 2017**).

BP also includes coenzymes and enzymes including diastase, phosphatase, amylase, catalase, saccharase, pectase, lactic dehydrogenase, lipase, and diaphorase in addition to AAs (**El-Neney and El-Kholy, 2014**). Additionally, ribonucleic acid, in particular, is present in substantial proportions in BP (**Komosinska-Vassev et al., 2015**).

Essential fatty acids should be highlighted first among the lipids (fatty acids, sterols, triglycerides, and phospholipids) that make up roughly 5.1% of blood plasma. Depending on the plant species, the FA concentration of BP ranges from 1 to 20% (**Brodshneider and Crailsheim, 2010**).

Phospholipids make up 1.5% of the total, while phytosterols, particularly *P*-sitosterol, make up 1.1% of the whole (**Szczęśna, 2006**).

According to recent research, BP provides a reliable source of PUFAs, which are essential for human nutrition. PUFAs must be taken from food because they cannot be produced by the human body on its own. Accordingly, BP may serve as a source of PUFAs in the diet of humans (**Taha, 2015; Kaplan et al., 2016**).

When examining the FAs composition of different forms of pollen (sunflower pollen, comparing hand-, bee-, and stored pollen, i.e. bee bread), **Nicolson and Human (2013)** discovered very little differences. There were no variations in FA composition between the groups in their investigation. Lauric acid was found to be the most prevalent FA in sunflower pollen, followed by palmitic and -linolenic acids.

Bees mix honey and bee secretions to pollen to provide a protein-rich food supply for the hive, giving bee bread a somewhat different composition (**Sammataro and Avitabile, 2011**).

18 FAs were found in bee bread that came from an indigenous bee plant in South Africa, according to **Human and Nicolson (2006)**.

22 FAs were found in the bee bread (containing >45% rape or willow pollen) that was gathered in the spring and summer by **Čeksterytė et al. (2008)**. The most prevalent unsaturated FAs were (Z)-octadec-9-enoic and (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acids, which together made up around 15% of all FAs.

In a different investigation, **Čeksterytė and Jansen (2012)** found that among 22 FAs found in spring rape and willow bee bread, (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (-3) had the greatest amount (27 - 43.8%).

Minerals and vitamins are among the beneficial components found in BP. Provitamin A (-carotene), vitamins E and D, and water-soluble vitamins such the B-complex, vitamin C, rutin, lecithin, and inositol are all found in a large amount in BP (0.1%) and (0.6%), respectively. They make up a total of 0.7% of the entire product. Macroelements including Ca, P, Mg, Na, and K as well as microelements like Fe, Cu, Zn, Mn, Si, and Se are all present in roughly 1.6% of the material. 0.02% of the latter one is present (**Komosinska-Vassev et al., 2015**).

The concentrations of particular macro- and microelements in BP with a specific floral origin, harvested in Romania, were analyzed by **Stanciu et al. (2012)**. These authors reaffirmed that BP is a natural source of minerals that are crucial for good nutrition. According to the authors' research, Fe and Zn were the leading microelements whereas K, Ca, and Mg were the prominent macroelements.

According to study of **Taha (2015)**, the date palm has the highest concentrations of Mg, P, and Mn in relation to its botanical origin, Ca and Zn for alfalfa, Cu for sunflowers, Fe for summer squash, and Na and K for rape.

Numerous investigations have shown that BP contains phenolic chemicals in addition to vitamins. The main components of BP's phenolic makeup are hydroxycinnamic acids and flavonol glycosides. This composition is usually species-specific and has been connected to BP's healing abilities. Differences in the qualitative and quantitative composition of BP phenolic compounds can be attributed to factors such as floral origin, geographic collection location, and bee species (**Sun et al., 2017**).

According to **Perez-Pérez et al. (2012)**, flavonoids are considered to be the primary component substances that indicate the quality of BP and can be utilized to establish quality criteria in respect to their nutritional-physiological characteristics.

Leukotrienes, catechins, and phenolic acids are some more phenolic molecules (**Komosinska-Vassev et al., 2015**).

According to **Freire et al. (2012)**, BP from Brazil contained the flavonoids isoquercetin, myricetin, tricetin, quercetin, luteolin, selagin, kaempferol, and isorhamnetin.

p-hydroxycinnamic acid, dihydroquercetin, isorhamnetin, isorhamnetin-3-O-(6''-O-E-pcoumaroyl)-D-glucopyranoside, luteolin, and quercetin were also listed as being present in BP in another Brazilian study (**Silva et al., 2009**).

In the free and bound phenolic extracts of rape BP, **Sun et al. (2017)** found 12 and 9 phenolic acids, respectively. The highest concentrations of rutin, benzoic acid, resveratrol, and quercetin were discovered in their investigation.

In samples of BP obtained in the Baltic region, research by **Kaškonienė et al. (2014)** found the presence of 2-hydroxycinnamic acid, rutin, and quercetin. In 13 of the 14 samples, naringenin was found. Additionally, ferulic, gallic, and caffeic acids were found in a few samples. Their research shown that the radical scavenging ability of BP extracts exhibits a high correlation with the total concentration of phenolic components.

Scientists have been able to pinpoint the BP's antimicrobial, antifungal, antioxidant, antiradical, anticancer, hepatoprotective, chemoprotective, and anti-inflammatory activities thanks to the scientific studies that have so far highlighted a variety of the substance's beneficial therapeutic and nutritional properties (**Pascoal et al., 2014a**).

Phenolic chemicals are said to be in charge of the biological activities (**Kaškonienė et al., 2014**). According to research by **Premratanachai and Chanchao (2014)**, polyphenols contained in BP have antiproliferative properties that can control cell division and trigger apoptosis.

Since it has been noted that BP exerts metal chelation capabilities and that can diminish the markers of oxidative stress (**Abdella, Tohamy and Ahmad, 2009**), BP may be utilized to boost immunological response, reduce the effect of radiation, and delay aging (**Babaei et al., 2016**).

Additionally, it has been asserted that BP has positive impacts on human health and prevents tumors, arteriosclerosis, prostate issues, and allergy desensitization. According to reports from **Nogueira et al. (2012)**, BP increases the pace of mitosis, encourages tissue regeneration, improves higher toxic removal, and lowers excessive cholesterol levels.

Additionally, it has been demonstrated that BP has the ability to block enzyme activity. Eucalyptus sp. and multiflora extracts of BP were shown by **Araújo et al. (2017)** to have strong inhibitory effect against the enzymes -amylase, acetylcholinesterase, tyrosinase, lipoxygenase, lipase, and hyaluronidase. Miconia sp. showed greater antihaemolytic activity in their investigation, while extracts of Cocos nucifera and Miconia sp. showed significant antioxidant capabilities in the other assays (ABTS, DPPH, -carotene/linoleic acid, and reducing power).

In this situation, BP has the potential to replace chemical medications in the treatment of several disorders. Its effectiveness as an anti-inflammatory agent has been demonstrated by

its capacity to block the hyaluronidase enzyme and to alleviate the overall signs and symptoms of inflammatory illnesses without causing any negative side effects (**Pascoal et al., 2014a**).

Additionally, a large number of studies have found that BP supplementation in broiler chickens has positive effects, particularly on performance and internal environment (**Wang et al., 2007; Hashmi et al., 2012; Kačániová et al., 2013; Attia et al., 2014; Farag and El-Rayes, 2016**).

Bee pollen applications for poultry

As information on meat performance, meat composition, and meat quality is covered in detail in the chapter Results and discussion, a concise summary of potential applications of BP in poultry is provided below.

In compared to birds fed diets containing either propolis or BP administered alone, **Kleczek et al. (2012)** found greater values of geometric parameters in the tibial bones of broilers fed a diet supplemented with the combination of the two substances.

According to **Oliveira et al. (2013)**, broilers fed feed supplemented with 1.5% BP until 21 days of age had improved immunity.

According to the results of a study by **Wang et al. (2007)**, BP may help broiler chickens' digestive systems develop early.

BP supplementation significantly enhanced the quantity of *Lactobacillus* and *Enterococcus* species in chicken caecums, according to another study (**Kačániová et al., 2013**), and might therefore be employed as a feed addition with prebiotic activity.

Following food supplementation with BP, **Kročko et al. (2012)** noted a decrease in the amount of Enterobacteriaceae family bacteria isolated from the crop, ileum, and caecum of broiler chickens, which may be related to BP's antibacterial action.

According to **Hosseini et al. (2016)**, broilers may benefit from the dietary use of BP (20 g.kg⁻¹) in high temperature environments to combat the negative effects of heat stress, with positive effects on intestinal morphometry, haematological profile, and heat stress biomarkers.

In a further investigation, broilers fed diets enriched with BP at rates of 0.2, 0.4, or 0.6% displayed better blood parameters (**Farag and El-Rayes, 2016**). Additionally, broiler hens given BP supplementation (300 mg.kg⁻¹) in the study by **Attia et al. (2014)** showed a rise in red blood cells and hemoglobin and a decrease in triglycerides, cholesterol, urea-N, creatinine, and aspartate aminotransferase.

Babaei et al. (2016) found that Japanese quails fed diets with 1,000 mg.kg⁻¹ of ethanolic extract of BP had the lowest ratio of heterophils to lymphocytes in their blood.

According to **Popiela-Pleban et al. (2012)** study, BP had a beneficial effect on the immune system of laying hens. This is probably because BP contains vitamins, minerals, and flavonoids.

According to research by **Arafa et al. (2016)**, BP, bee bread, and their combinations can be utilized as antioxidants in hen diets to improve production and interior egg yolk quality.

The findings of the study by **Yılmaz, Tath Seven and Kaya (2017)** showed that BP supplementation to quail's meal decreased the yolk lipid peroxidation significantly more than that of royal jelly. This might be because BP has a more potent antioxidant capacity than royal jelly. Additionally, adding BP to the diet increased the shelf life of eggs.

1.5.3 Propolis

Honeybees (*Apis mellifera* L.) gather propolis, also known as bee glue, naturally from a variety of plants, mainly from flowers and leaf buds, and carry it back to the colonies (**Babaei et al., 2016**). According to **Pasupuleti et al. (2017)**, the word "propolis" is derived from the Greek words "pro" (defence) and "polis" (city or community), which together refer to the beehive.

According to **Huang et al. (2014)**, the material collected is typically made up of resins from poplars, conifers, birch, pine, alder, willow, palm, *Baccharis dracunculifolia*, and *Dalbergia ecastaphyllum*. It can also be a wound exudate (resin and latex) or a secretion (lipophilic compounds, mucilage, and gum). Thus, propolis comes from a considerably wider range of sources than any other substance that honeybees gather.

The final product was created by honeybees who combined it with the salivary enzyme -glycosidase, partially digested it, and then added it to bee wax (**Pascoal et al., 2014b**). In order to protect the colony and larvae from pathogenic microorganisms, such as virus, bacteria, and fungi, particularly *Bacillus subtilis*, *B. alvei*, *Proteus vulgaris*, and *P. galangin* agent, propolis is then used in the construction and adaptation of beehives during the building of combs (**Aygun, Sert and Copur, 2012**).

It is also used to smooth the inside of the beehive, maintain the internal temperature of the hive (35 °C), and stop weathering and predator invasion. Propolis also adds to an aseptic interior environment by hardening the cell wall (**Pasupuleti et al., 2017**).

The chemical makeup of propolis is very intricate. According to **Zingue et al. (2017)**, more than 300 chemical components have been found in raw propolis. Plants, chemicals released during honeybee metabolism, and materials added during propolis synthesis are the three potential origins of the organic compounds in propolis (**Al-Ghamdi et al., 2017**). According to **Huang et al. (2014)**, raw propolis typically contains 50% plant resins, 30% waxes, 10% essential and aromatic oils, 5% pollens, and 5% other organic materials.

Table 6: The average chemical composition (some basic components) of different colour types of propolis collected in different regions of Brazil (**Machado et al., 2016**)

Parameter (%)	Red	Green	Brown
Moisture	7.15	8.33	7.71
Total ash	1.00	3.23	1.65
Protein	2.01	10.13	3.88
Lipids	66.54	47.27	65.97
Fibre	5.55	17.72	25.60

In addition to polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, and ketones), propolis also contains terpenoids, sesquiterpene quinones, coumarins, steroids, amino acids, fatty acids, inorganic compounds, and sugars (**Da Silva Frozza et al., 2013; Babaei et al., 2016**).

Table 7: Fatty acid composition of propolis (**Rebiai et al., 2017**)

Fatty acid	Amount (%)
C20:1 n-9	8.14
C20:2 n-6	7.53
C18:2 cis n-6	5.11
C20:5 n-3	4.86
C20:4 n-6	3.81
C16:1	3.87
C16:0	1.07
C18:3 n-3	0.40
SFA	2.42

MUFA	12.72
n-6 PUFA	21.97
n-3 PUFA	0.57
n-6/n-3	92.21

Notes: Data are averaged from eight propolis samples of different geographical origin. C20:1 n-9 = eicosenoic acid; C20:2 n-6 = eicosadienoic acid; C18:2 cis n-6 = linoleic acid; C20:5 n-3 = eicosapentaenoic acid; C20:4 n-6 = arachidonic acid; C16:1 = palmitoleic acid; C16:0 = palmitic acid; C18:3 n-3 = α -linolenic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Aromatic acids, lactones, ketones, phenols, nucleic acids, aldehydes, and cinnamic acid and its derivatives are among the other biologically active substances (**Gutiérrez-Cortés and Mahecha, 2014**). Depending on the botanical and geographic characteristics, as well as the collecting season, their abundance and the overall chemical composition of propolis, are qualitatively and quantitatively vary (**El-Bassuony, 2009**).

Similar to how color changes depending on location and plant sources, it ranges from light yellow to dark brown. Additionally, propolis has different physical properties depending on the temperature; it is hard and brittle between 0 and 15 °C, pliable and mushy between 30 and 60 °C, sticky beyond 60 °C, and liquefies above 70 °C (**Açikgöz, Yücel and Altan, 2005**).

Propolis frequently contains polysaccharides, such as starch, as well as the di- and monosaccharides glucose, fructose, ribose, rhamnose, talose, gulose, and saccharose (**Kurek-Górecka et al., 2013**).

Additionally, propolis is high in minerals (Ca, Cu, I, K, Mg, Na, Zn, Mn, and Fe), vitamins (B₁, B₂, B₆, A, C, and E), and enzymes (maltase, esterase, and transhydrogenase) (**Khan, 2017**).

The vast array of pharmacological activities of propolis, which may be the result of the synergistic action of its complex constituents, particularly the aromatic acids, diterpenic acids, flavonoids, and phenolic compounds, suggest that it is widely used in medical science, apitherapy, and bio-cosmetology (**Mahmoud, Cheng and Applegate, 2016**). Propolis's characteristics are greatly influenced by the plant sources that honeybees consume (**Aygun, 2017**).

Propolis' overall polyphenol and flavonoid content is one of the most crucial factors to consider when assessing its biological potential (**Pascoal et al., 2014b**). The majority of the components in propolis, between 45 and 55%, are flavonoids and phenolic acids or their esters (**Aygun, 2017**).

Regarding the phenolic and flavonoid compounds, some studies revealed that caffeic acid (CA) and caffeic acid phenethyl ester (CAPE) is a biologically active ingredient of propolis with several interesting biological properties, including enhancing apoptosis (**Draganova-Filipova et al., 2008**), reducing metastasis (**Liao et al., 2003**) and increasing radiation sensitivity of cancer cells (**Chen et al., 2005**).

Chrysin and propolins are two more anticancer compounds found in propolis in addition to CA and CAPE (**Maruta and Ohta, 2009**). New substances have recently been isolated from propolis, such as the artemillin C (3,5-diprenyl-4-hydroxycinnamic acid) from Brazilian green propolis, which has been shown to be a key immunomodulatory substance (**Cheung et al., 2011**) as well as the main anti-cancer component of this propolis (**Maruta and Ohta, 2009**).

According to reports, this product has a variety of pharmacological properties, including those that are antibacterial (**Oliveira et al., 2017**), antiviral (**Schnitzler et al., 2010**), antioxidant (**Ahmed et al., 2017**), anti-inflammatory (**Almeida and Menezes, 2002**), anticancer (**Amini-Sarteshnizi et al., 2015**), hepatoprotective (**Banskota et al., 2001**).

Additionally, it has been demonstrated to be useful in lowering the risk of atherosclerosis (**Nader, El-Agamy and Suddek, 2010**), in encouraging tissue regeneration, and in increasing the removal of harmful substances (**Nirala and Bhadauria, 2008**). Propolis can lower blood sugar levels in experimental diabetic rats, modify the metabolism of blood lipids, resulting in less lipid peroxidation, and scavenge free radicals, according to **Al-Hariri (2011)**.

Propolis application may help improve oral health due to its antibacterial activity against a wide range of Gram-positive pathogenic microorganisms and limited action against Gram-negative pathogenic microorganisms (**Akca et al., 2016**), as well as for protecting various agricultural products during storage as an alternative preservative agent (**Aygun, Sert and Copur, 2012**). Propolis' ability to effectively seal the pores of the eggshell has been shown to increase the life of the shell of table eggs, as shown by **Copur et al. (2008)** and **Sahinler, Gul and Copur (2009)**, among others.

According to studies, adding propolis to the diet has a positive impact on a variety of quality and health-related indicators, including growth performance, carcass characteristics, egg production and quality, meat composition and quality, immune response, and many others (**Attia et al., 2014; Daneshmand et al., 2015**). The action of propolis on the GI microbiota, which increases the quantities of helpful bacteria and decreases the pathogenic types, may be responsible for these benefits (**Kačániová et al., 2012**).

Propolis applications in poultry

The literature on propolis applications in poultry with an emphasis on factors other than meat performance, meat composition, and meat quality will be reviewed briefly below.

In laying hens, propolis supplementation (250 and 1,000 mg.kg⁻¹) significantly increased egg mass, egg production rate, shell weight, Haugh unit, albumen height, and yolk index, according to study by **Abdel-Kareem and El-Sheikh (2015)**. A further study (**Galal et al., 2008**) found that laying hens fed diets with 100 and 150 mg propolis likewise produced eggs with the heaviest mass when compared to the control group.

In terms of the immune system, **Galal et al. (2008)** found that laying hens supplemented with 100 and 150 mg of propolis had significantly higher lymphocyte counts and significantly fewer heterophils than the control group.

According to a study by **Ziaran, Rahmani and Pourreza (2015)**, broiler chicken immunological responses were positively impacted by low doses of propolis oil extract (40 and 70 mg.kg⁻¹), but negatively impacted by high doses (400, 700, or 1,000 mg.kg⁻¹).

Abdel-Kareem and El-Sheikh (2015) found that laying hens given diets containing 250, 500, and 1,000 mg propolis.kg⁻¹ had significantly lower heterophils counts and significantly higher lymphocyte counts than the control group.

According to **Babaei et al. (2016)**, Japanese quails' antibody titre and lymphoid organ weight rose when they were given an ethanolic extract of propolis (5,000 mg kg⁻¹).

Since it has been shown that injecting propolis (epimedium polysaccharide-propolis flavone immunopotentiator) could increase antibody titre and IgG, IgM, IFN- and IL-6 concentrations, promote lymphocyte proliferation and increase immune organ index in immunosuppressive chickens induced by cyclophosphamide, study by **Fan et al. (2013)** suggested that propolis could be used in prevention and treatment of animal immunosuppressive diseases.

According to **Cetin et al. (2010)**, adding propolis to the feed of laying hens at a dosage of 3000 mg.kg⁻¹ increased serum IgG and IgM levels while lowering the percentage of T lymphocytes in the peripheral blood when compared to controls.

Furthermore, the study by **Biavatti et al. (2003)** clearly demonstrated a compensatory gain in chickens receiving propolis supplements after being challenged with *Eimeria acervulina* oocysts on the seventh day. This gain may be attributable to the propolis extract's antimicrobial activity, which improves digestion and absorption and results in better intestinal health.

According to a study by **Mahmoud et al. (2015)**, adding green Brazilian propolis to male broiler feed reduced the behavior associated with heat stress, particularly when given in dietary doses of 250 or 3,000 mg.kg⁻¹. This was accomplished by enhancing bird movement activities and decreasing panting behaviors.

According to results of a different study (**Mahmoud et al., 2017**), adding green Brazilian propolis to the diet at the tested doses (100, 250, 500, 1,000, or 3,000 mg.kg⁻¹) improved the health status of male broilers raised under mild chronic heat stress by reducing the onset of heat stress responses, such as decreased concentrations of corticosterone, aminotransferase, and uric acid.

Tekeli et al. (2010) showed that feeding male broilers propolis extract (1,000 mg.kg⁻¹) as a dietary supplement might decrease the amount of coliform bacteria, accelerate the growth of lactic acid bacteria, and lengthen the intestinal villi.

According to a study by **Attia et al. (2014)**, broiler chickens had higher levels of red blood cells and hemoglobin and lower levels of triglycerides, cholesterol, urea-N, creatinine, and aspartate aminotransferase.

1.5.4 Plant residues and by-products

Consumer concerns about the harmful effects of antibiotic use and the subsequent ban on antibiotics in the EU have prompted researchers to explore different alternatives to antibiotics (**Diarra and Malouin, 2014**). The purpose of these alternatives is to maintain a low mortality, a good level of meat yield of the animals while preserving the environment and the health of consumers. Much research has been done to find natural substances with similar beneficial effects as the growth stimulants used. In fact, there are several non-therapeutic alternatives that can replace the use of antibiotics in the fattening process of farm animals.

Among them, probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocins, bacteriophages, phytogetic feed additives, phytocides, nanoparticle essential oils and plant processing residues appear to be the best (**Mehdi et al., 2018**).

Due to population growth, more food production is also needed to improve global food security (**Hartikainen et al., 2018**), so those involved in the nutrition of animals intended for human consumption must optimize the efficiency of livestock rations, i.e. obtain more meat, milk and eggs (**Makkar, 2016**).

It is also necessary to limit and reduce the impact of livestock on the environment, use less land, water, energy and fertilizers (Röös et al., 2017) and reduce greenhouse gas emissions (Salami et al., 2019).

A possible strategy to achieve these goals is to use agricultural waste, such as plant residues or by-products of the food industry (Ulloa et al., 2004) to feed livestock destined directly or indirectly for human consumption (Westendorf et al., 1996).

Based on the demand for food of animal origin for human consumption, production systems require a shift towards more sustainable livestock farming, which is based on the efficient use of available food resources, waste reduction and the use of new nutritional sources of feed, especially those not intended directly for people (Wadhwa and Bakshi, 2013).

In this context, plant residues are the key to achieving this goal. The use of plant waste to feed livestock is actually not new as it is a common practice in rural areas (Angulo et al., 2012).

Incorporation of plant wastes into livestock feed has the potential to reduce production costs (Wadhwa and Bakshi, 2013). This is particularly important as feed is one of the most expensive items in livestock farming. However, it should be emphasized that the use of plant residues in animal feed does not always mean a reduction in production costs, as their processing alone can disproportionately increase production costs. For example, agricultural residues, due to their high moisture content, require long-term drying, which is energy-intensive.

The environmental benefit is mainly considered to be a reduction in the amount of waste that ends up in landfills (Cook et al., 2018) and a lower abundance of insects for which plant waste is an ideal breeding ground (Beausang, Hall and Toma, 2017).

Currently, there is no estimate of the amount of agricultural waste produced, mainly due to the absence of records and the fact that harvest periods and the amount of harvested crops are different every year and the waste has no economic value as it does not find use in any market (Röös et al., 2017).

However, the FAO notes that one-third of all food produced is thrown away (Beausang, Hall and Toma, 2017), with the highest proportion being fruit and vegetable waste (Gustavsson et al., 2012).

Large losses of food already occur in the phase of post-harvest storage and processing (Giroto, Alibardi and Cossu, 2015).

China is one of the countries that produces the most plant waste, producing approximately 32 million tons per year, while the US produces 15 million tons, which are at best used for composting or simply dumped in landfills (**Wadhwa and Bakshi, 2013**).

The use of plant waste as a soil fertilizer or as a raw material for the production of biofuels (**Beausang, Hall and Toma, 2017**) and as an alternative feed for farms is also reported (**Hartikainen et al., 2018**).

In this way, plant waste can continue to be part of the food chain (**Beausang, Hall and Toma, 2017**), because farm animals, compared to humans, are able to use nutrients from this type of waste by converting them into nutrients contained in the final product (meat, milk, eggs, etc.).

Thus, the use of by-products of crop production as feed for livestock prevents competition between food and feed (**Salami et al., 2019**) as well as contributes to the efficient use of local agricultural resources (**Röös et al., 2017**).

The main problem with the use of agricultural by-products is the high moisture content, which makes handling difficult and promotes their rapid decomposition. Therefore, it is necessary to use a certain form of conservation of this waste (**Ulloa et al., 2004**), without reducing their quality (**Esteban et al., 2007**).

In addition, it must be taken into account that plant residues are not produced constantly throughout the year, so that most of the production is only available during certain months of the year (**Acosta-Martínez, Avendaño-Ruiz and Astorga-Ceja, 2015**).

Consequently, plant residues could be an alternative feed during the dry season when other resources are exhausted (**Valbuena et al., 2015**).

Silage is an option that could be used to preserve plant residues for a longer time (**Hossain et al., 2015; Chavira, 2016**), but plant residues due to

due to high moisture content, it cannot be ensiled alone, so they are mixed with straw or hay (**Wadhwa and Bakshi, 2013**).

Plant residues contain various bioactive compounds such as vitamins, unsaturated fatty acids and phytochemicals that can be beneficial for animal health and productivity (**Salami et al., 2019**).

Several studies have shown that plant residues can be used as part of livestock nutrition, 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 skins, pulp, and seeds of fruits and vegetables are a source of polyphenols, which have anticancer, antimicrobial, antioxidant, and immune system-stimulating properties. Essential oils, which are obtained from the peel of some citrus fruits, can extend the shelf life of food. Plant residues can also contain antioxidants, which act by eliminating free radicals and preventing the formation of peroxides. In addition, plant residues can be a source of various enzymes such as bromelain (pineapple), papain, amylase, laccase, and manganese peroxidase, which have various biological and biotechnological uses (**Wadhwa and Bakshi, 2013**).

Flax applications

According to **Jheimbach and Port Royal (2009)**, flaxseed is a complex system made up of bioactive plant materials like fats with a desirable fatty acid 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). Table 8 displays the flaxseed's basic constitution.

Table 8: 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		

Flax is used in animal diets in a variety of ways, including whole seeds, meal, hulls, and oil supplements. The byproduct of seed oil extraction is meal, sometimes referred to as linseed cake in Europe and Asia. Both ruminants and non-ruminants can benefit from the addition of this beneficial feed product to their diets (**Jhala and Hall, 2010**).

Flax seed meal contains about 38% hull, which is twice as much as canola or soybean meal (**Agriculture and Agri-Food Canada, 1997**). The medium and mix fractions can be incorporated into formulas for chicken feed, while the fine fraction obtained as a byproduct of dehulling (a process of preparing flaxseed for value added industrial goods) may be used in pet food (**Oomah, Kenaschuk and Mazza, 1996**). Dogs, cats, and horses' mixed pet diets all contain flax seed oil. The essential fatty acids (ALA and LA) in flax seed assist animals have lustrous coats, reduce digestive and skin issues, and prevent dry skin and dandruff (**Jhala and Hall, 2010**).

By feeding laying hens 10 – 20% more ground flax seed, omega-3 enriched eggs are produced. The amount of omega-3 fatty acids in the eggs produced with this feeding formula would be ten times higher than in regular eggs (**Canadian Egg Marketing Agency, 2007**). One omega-3 enriched egg supplies roughly a quarter of the recommended daily intake of EPA and DHA and about half of the recommended daily consumption of ALA (**de Lorgeril et al., 1999**).

Linseed-based n-3 PUFA feeding to pigs enhances the nutritional value of pork. Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) concentration in muscle and adipose tissue were examined in a meta-analysis including 1006 pigs published in 24 articles. The following changes in muscle fatty acid composition were observed as a result of n-3 PUFA: ALA + 137%, EPA + 188%, DPA + 51%, and DHA + 12%. The same outcomes – ALA + 297%, EPA + 149%, DPA + 88%, and DHA + 18% – were seen in adipose tissue. It was found that dietary therapy and the amount of ALA and EPA in muscle and adipose tissue were positively correlated. DPA and DHA were shown to be significantly correlated with live weight in muscle. By increasing the level of n-3 PUFA in muscle and adipose tissue, feeding linseed to pigs increased the nutritional quality of pork (**Corino et al., 2014**).

Flax preparation for feeding by successfully transferring dietary ALA into milk with noticeable increases in the n-3 to n-6 FA ratio, LinPro to cows at 23 g/kg and 47 g/kg of diet increased the milk's health-related quality for its human consumers. The overall rise in milk production as the dietary level of LinPro increased was caused, at least in part, by the increased diet net energy for lactation density in both LinPro-fed groups, which suggested more effective

use of dietary energy for milk production. For cows fed the flaxseed preparation LinPro, there was also an improvement in general health (i.e., lower somatic cell count and mastitis culling) **(Swanepoel and Robinson, 2019)**.

A wide range of advantages for human health are provided by PUFA of the n-3 family consumption, including enhanced cardiovascular and mental function. The first line of n-3 enriched food products to enter the market effectively was probably eggs. Due to its high fat (>38%) and ALA (>50%) concentrations as well as other nutritional qualities (such protein and metabolizable energy), flaxseed is the most often used feed item investigated for egg n-3 fatty acid enrichment. Even a 1% increase in the laying hen diet increased the amount of n-3 fatty acids in each egg by 40 mg. Over 440 mg of ALA and 170 mg of long-chain n-3 fatty acids can be obtained by eating 2 eggs from chickens fed 10% flax. For many civilizations around the world, chicken eggs are one of the most widely consumed and reasonably priced foods. One natural, effective, and sustainable strategy to address the human need for n-3 fatty acids is by using flax in the meals of layer hens and generating eggs rich in n-3 fatty acids **(Cherian, 2017)**.

Milk's concentration of PUFA and c9t11-conjugated linoleic acid (CLA) rose when false flax (*Camelina sativa*) pomace was added to dairy goat diets. When compared to kefir made from goat milk fed a basal diet, kefir from goat milk fed a CS cake supplement exhibits a significantly higher amount of bioactive components (PUFA, including CLA) in the fat fraction. Kefir made from the milk of nanny goats from both feeding groups had the same basic chemical makeup and flavor, aroma, and consistency **(Pikul et al., 2014)**.

Generally speaking, dietetic enrichment with flax seed pomace, 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, and p-coumaric), anthocyanin.

Pumpkin applications

Pumpkin seeds are a great source of protein, good quality oil, and nourishment. They also have pharmacological properties that include anti-diabetic, anti-fungal, anti-bacterial, anti-inflammatory, and antioxidant actions. In the past, they were used to make oil, strengthen breads, serve as a snack, and even as a medicine. According to **Ningthoujam, Prasad and Palmei (2018)**, pumpkin seeds are nutritional powerhouses with a wide range of nutrients and have numerous health advantages. Table 9 lists the basic nutritional makeup of the pulp, skin, and seeds of the three most prevalent pumpkin species.

Table 9: 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	<i>Cucurbita pepo</i>	<i>Cucurbita moschata</i>	<i>Cucurbita maxima</i>
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

According to research, pumpkins are traditionally and sparingly used to feed a variety of domestic animals, including ruminants and horses (Lans et al., 2007; OECD, 2016). According to studies on the use of pumpkins in animal feeding, its benefits for productivity are due to the fruit's high amount of carbs, minerals, and vitamins as well as protein and fat in the seeds (Achilonu et al., 2017).

According to Dorantes-Jiménez et al. (2016), when it comes to the fruit, *Cucurbita argyrosperma*'s dry residue (peel and pulp only) has a low protein content (9%), but it also contains nearly 50% neutral detergent fiber and 40% acid detergent fiber, making it suitable for the dietary preparation of dairy cattle and rabbits. Contrarily, Crosby-Galván et al. (2018) note that replacing up to 30% of corn stubble with dry residue from *Cucurbita argyrosperma* increases the ruminal digestibility of dry matter by 21%; however, this decreases the digestibility of neutral detergent fiber by 7%, which is attributed to the degradation of nonfibrous carbohydrates like sugars, which are quickly fermented. Pumpkin's sugar content

may be one of the factors contributing to the diet's improved palatability. Pumpkin fruits can also be used to make silage, but only if they are combined with other substances that have less moisture, like 20% beet pulp. By supplying readily fermentable carbohydrates in this instance, beets enhance the silage's properties (**Łozicki et al., 2015**).

There aren't many studies on the feeding of ruminants with pumpkins as of yet. When 17% of corn silage was replaced with *Cucurbita maxima* silage in cattle, Halik et al. (2018) found that milk production increased by about 6 kg.day⁻¹ (20.7 kg.day⁻¹ vs. 26.5 kg.day⁻¹, respectively), but there was no difference in weight gain in buffalos when up to 14% of *Cucurbita pepo* silage was added (**Razzaghzadeh, Amini-Jabalkandi and Hashemi, 2007**).

Using pumpkin seed flour did not affect the laying rate or egg quality in laying hens (**Hajati, Hasanabadi and Waldroup, 2011; Martínez et al., 2012**). According to **Machebe et al. (2013)**, adding 5% of seeds to the diet of turkeys raised hatching rates, decreased embryonic mortality, and increased egg fertility.

A report by **Medina-González et al. (2019)** shows that weight gain in pigs is unaffected when up to 30% of the ration is substituted by *Cucurbita pepo* ferment, despite the paucity of studies on the use of pumpkins as food in pigs. However, there was a 75% rise in food intake, which had an impact on food conversion.

Pumpkins have a number of medical uses in addition to being used as food. In this regard, many chemicals with various biological activity have been found in seeds and fruit, including antioxidant, antifungal, antiparasitic, antibacterial, and anti-inflammatory properties (**Yadav et al., 2010; Achilonu et al., 2017**). Therefore, by include pumpkin in the diet, cattle health can be enhanced, which will also increase productivity and welfare. While *Histomonas meleagridis*, *Tetratrichomonas gallinarum*, and *Blastocystis* sp., protozoans of economic importance in poultry farming, have been shown to be resistant to *Cucurbita pepo* ethanolic extract in in vitro assays in birds, its effectiveness is limited in vivo (**Grabensteiner et al., 2008**). Additionally, it has been demonstrated that the lectins from pumpkin seeds have antibiotic effects against *Salmonella typhimurium*, *Salmonella gallinarum*, *Escherichia coli*, and *Pseudomonas*, which suggests that their use may reduce the need for antibiotics. The entire fruit also possesses antiviral properties against the New Castle virus (**Achilonu et al., 2017**). According to **Medina-González et al. (2019)**, the fermented form of *Cucurbita pepo* reduces the incidence of diarrhea in pigs, which lowers the mortality and morbidity of piglets. Pumpkins have most likely been employed as antiparasitic drugs (**Lans et al., 2007; Yadav et al., 2010; Achilonu et al., 2017**) because the cucurbitacins they contain typically have digestive and

purgative activity due to their bitter chemical concentration (**Montesano et al., 2018**). The high mucilage content of *Cucurbita moschata* peel extract, which lowers oxidative stress on burned tissue, has also been shown by **Bahramsoltani et al. (2017)** to be effective in treating burns.

As a valuable source of protein, pumpkin seed pomace – a byproduct of the extraction of pumpkin seed oil – is utilized in ruminant feed. **Li et al. (2021)**'s experiments evaluated the performance, rumen fermentation, antioxidant activity, and nitrogen partitioning of pumpkin seed pomace as an alternative to soybean meal in nursing cows' diets. The cows were separated into three treatment groups at random: one that did not get any treatment; one that did; and one that received dry distillers' grains with solubles at levels of 50 and 100%, respectively, in place of the soybean meal. The diets were isonitrogenous and contained the same amount of roughage, but varying amounts of dry distillers' grains with solubles and pumpkin seed pomace. The nitrogen partitioning between milk, feces, and urine did not differ in the animals fed the three diets, and the substitution of soybean meal with pumpkin seed pomace and dried distillers' grains with solubles had no effect on rumen degradation, milk performance, rumen fermentation, DM intake, or apparent total tract digestibility. However, the animals that received the 50% and 100% pumpkin seed pomace had higher total antioxidant capacities and antioxidant enzyme activities (total superoxide dismutase, glutathione peroxidase, and catalase) than the animals whose diets contained no pumpkin seed pomace. Pumpkin seed pomace, on the other hand, dramatically decreased the plasma levels of aspartate transaminase, alkaline phosphatase, and malondialdehyde. These findings show that soybean meal can be completely replaced by pumpkin seed pomace in the diet of dairy cows without having any negative effects on milk production, rumen fermentation, or apparent digestibility. Additionally, this dietary change enhances antioxidant functions and blood parameters in dairy cows, demonstrating the potential for PSP to be used as a feed source for dairy cows (**Li et al., 2021**).

Regarding alterations in the haemato-chemical parameters of lambs' blood in organic farming, partial replacement of soybean meal with pumpkin seed pomace maintained appropriate carcass features. Because *m. semimembranosus* contains higher levels of linoleic acid (LA) and has a higher ratio of LA to α -linolenic acid (ALA), replacing soybean meal with 10 and 15% of PSP exhibited less favorable effects. Accordingly, in terms of the haemato-chemical parameters and carcass features, pumpkin seed pomace could be employed in lamb feed as a substitute for soybean meal. Future studies must investigate PSP's antioxidant properties in lamb feed and get higher replacement levels (**Antunović et al., 2018**).

In a study by **Klir et al. (2017)**, the effect of replacing soya bean meal in goats' diets with PSP or extruded linseed (ELS) on milk yield, milk composition, and the fatty acids profile of milk fat was detected. In a 75-day study, dairy goats were split into three groups and fed concentrate combinations with soy bean meal ELS or PSC as the primary protein source. In contrast to the fatty acid profile, the addition of ELS or PSC had no effect on milk yield and milk gross composition when compared to the control group. In comparison to control and PSC, supplementing with ELS led to higher levels of total n-3 fatty acids and branched-chain fatty acids (BCFA). The ELS group's milk had higher proportions of ALA (ALA, and EPA) and total n-3 fatty acids. ELS and PSC, in contrast, led to decreased LA proportions when compared to control. As compared to control or PSC, the aforementioned resulted in a decreased LA/ALA ratio with ELS supplementation. Comparing the PSC diet to the control, total n-6 fatty acids were reduced. Soybean meal may be partially supplemented with ELS diets to boost beneficial n-3 fatty acids and BCFA while decreasing the LA/ALA ratio and increasing C18:0. According to **Klir et al. (2017)**, PSP totally replaced soya bean meal in the diet of dairy goats without causing any noticeable alterations in the fatty acid profile that would be relevant to commerce or human health.

Grape pomace applications

The wine industry generates a huge amount of waste, which consists of stems, grape pomace, sewage sludge and yeast sludge. The processing or disposal of winemaking waste can have a toxic impact on the environment (**Ilyas et al., 2021**).

According to **Mendes et al. (2014)** and **García-Lomillo and Gonzalez (2017)** about 1 kg of pomace is produced for every 6 l of wine. Traditionally, grape pomace is incorporated into the soil or fed to livestock.

Grape pomace can also be used for the production of a large number of products with added value, as they contain various organic acids (tartaric, malic and citric), alcohol, fiber and grape seed oil with a positive fatty acid composition (**Maier et al., 2008**).

In addition, grape pomace is a source of polyphenols, including flavonoids, anthocyanins, proanthocyanidins and phenolic acids (**Fontana et al., 2013; Beres et al., 2017; Garcia-Lomillo and Gonzalez, 2017; Del Pino-García et al., 2017**).

After the production of wine, the subsequent process of valorization of by-products of production is aimed at their reuse, for example, in products with added value or as raw materials

for other industries, which should result in a reduction of negative impacts on the environment. Therefore, effective methods are being sought for the extraction of valuable substances from grape pomace, such as polyphenols in particular. A good example of the recovery of grape pomace is, for example, obtaining grape seed oil with an excellent composition of fatty acids, which is quite common today. It is the grape seeds that are difficult to decompose compared to the skins, which is why the production of this oil makes the entire wine production process more sustainable (**Environmental Protection Agency, 2015**).

Various antifungal, antimicrobial, anti-inflammatory, anti-cancer and cardioprotective effects have been observed in polyphenol-rich grape pomace extracts (**Ky et al., 2014**).

In the topic of valorization of winemaking by-products, there is room for further reduction of adverse effects on the environment by supporting the commercialization of grape oil and various foods enriched with grape pomace and their extracts (pasta, biscuits, whole grain bread, etc.) (**Alexandre et al., 2018**).

A considerable amount of waste is produced during the cultivation of the vine itself and during the production of wine. Waste from the wine industry can be characterized as solid and liquid. Solid waste from the wine industry is generated as a result of the harvesting and processing of grapes, while liquid waste is more likely to be generated during the winemaking process. Collectively, waste in the production of grapes and vines mainly consists of stalks, seeds, skins, yeast sludge, organic acids, CO₂, branches when cutting vines and polluted water. In the best case, these are used for the production of products, such as fertilizers, feed, or as a raw material intended for further processing (**Rondeau et al., 2013**).

Solid waste mainly consists of grape stalks, which are separated separately, and grape pomace consisting of skins and seeds, which differ in texture and chemical composition, especially depending on the variety. They are used either together or separately, especially if oil is obtained from the kernels (**Haščik et al., 2020**).

Broome and Warner (2008) reported that percentage amounts of wine industry waste consist of approximately 45% grape skins, 7.5% grape stems, 6% grape seeds and other wastes.

With a production of approximately 5 tons of grapes per hectare per year, grape pomace is the main by-product of the vineyard (**Barrantes Leiva, Hosseini Koupaie and Eskicioglu, 2014**).

They have agronomic value and contain a large amount of cellulose, lignin, sodium and potassium. Due to the low content of organic matter (2 – 3%), grape stalks are used for composting and subsequent incorporation into the soil (**Nerantzis and Tataridis, 2006**).

Table 10: Proximate composition of grape pomace (based on dry matter) (**Antonić et al., 2020**)

Component	Content g.100 g ⁻¹	Component	Content mg.100 g ⁻¹
Ash	1.73 – 9.10	Na	87 – 244
Protein	3.57 – 14.17	K	1184 – 2718
Fat	1.14 – 13.90	Mg	92 – 644
Total fiber	17.28 – 88.70	Ca	91 – 961
Insoluble fiber	16.44 – 63.70	Mn	6 – 1356
Soluble fiber	0.72 – 12.78	Fe	5 – 5468
Total carbohydrates	12.20 – 40.53	Zn	2 – 2254
Total polyphenols	0.28 – 8.70	Cu	39 – 130
Fructose	0.38 – 8.91	P	4 – 3157
Glucose	0.21 – 26.34		

Antibiotics are commonly used not only to treat but also to prevent bacterial diseases in industrial livestock farming. Emerging resistant strains of bacteria, as well as antibiotic residues in meat and various animal products, have led to a reduction in their use (**Wang et al., 2017**) and, since 2006, to their complete ban in the European Union (**Pavelková et al., 2020**).

The global strategy also leads to a reduction in the use of antibiotics and other drugs in farm animals, and therefore various products rich in bioactive compounds with antimicrobial, antioxidant and anti-inflammatory properties are sought. These natural feed additives are promising alternatives to antibiotics (**Niewold, 2014; Lillehoj et al., 2018**).

Large and available quantities and amount of bioactive compounds present in grape pomace makes them a suitable candidate for use in animal nutrition (**Aditya et al., 2018**).

The beneficial effects of enriching poultry nutrition with grape pomace have been demonstrated in several experiments (**Kara et al., 2016**). For example, fortification with grape pomace as a source of antioxidants at 30 g.kg⁻¹ (**Brenes et al., 2008**) and 60 g.kg⁻¹ (**Goñi et al., 2006**) reduced lipid oxidation in broiler chicken meat.

Viveros et al. (2011) reported that the addition of grape pomace to the diet increased the amount of beneficial bacteria in the intestines of broiler chickens. **Hasčík et al. (2020)** recorded significantly higher some parameters of meat efficiency compared to the control

group, such as live weight and yield of valuable meat parts after the application of red grape pomace in the feed mixture of broiler chickens.

Čech et al. (2021) found a higher content of fat in the muscle of chickens, while the content of cholesterol, which is concentrated in fat, remained approximately the same compared to the control group of chickens. Subsequently, they observed an increase in the fat content of chicken meat after adding red grape pomace to their diet compared to the control group, but despite this, **Jurčaga et al. (2021)** in a follow-up study did not observe an increase in malondialdehyde (an oxidation signaling substance) in their meat, suggesting a possible protective antioxidant effect of grape pomace.

Ebrahimzadeh et al. (2018) found that the addition of grape pomace to a broiler feed mixture increased the immune response and reduced the feed cost per kg live weight.

Enrichment of pig feed mixture with fermented grape pomace did not affect production parameters, but suppressed some inflammatory cytokines in their liver. Their addition to the feed positively affected the color fastness of the meat, the content of polyunsaturated FA in the subcutaneous fat and its oxidative stability (**Taranu et al., 2018**).

In dairy cows, a higher concentration of PUFA in milk was observed after the addition of grape pomace. Furthermore, their digestive microflora was also positively affected, which was generally reflected in the better health of the animals (**Moate et al., 2014**).

2 AIM OF THE WORK

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

- a) meat performance (carcass weight, abdominal fat weight, percentage of abdominal fat from the carcass) of Ross 308 broiler chickens without and after application of different doses of various feed supplements to the feed mixture and water,
- b) 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 different feed supplements, which were used in complete feed mixture (FM) and water of broiler chickens of the hybrid combination Ross 308 in various quantities. Their effect on formation of abdominal fat in carcass of the Ross 308 broiler chickens was investigated. 11 experiments between years 2003 – 2022 were performed to achieve the aim of this scientific monograph.

3.2 Technical realization of the experiments

3.2.1 1st Experiment

The first experiment was carried out in the semi-operational conditions of the experimental facility in the Institute of Special Breeding at the SUA in Nitra. In the experiment, we monitored the effect of a probiotic preparation in liquid form applied through a water source on the formation of abdominal fat in Ross 308 chickens. 180 one-day-old chickens were included in the experiment, which were divided into 3 groups of 60 one-day-old chickens. Chickens were fed for 42 days with starter complete feed mixture (CFM) HYD-01 (1. – 21. days of fattening), growth CFM HYD-02 (21. – 36. day of fattening) and final CFM HYD-03 (37. – 42. day of fattening) (Table 11). The control group was without the application of probiotics and the experimental groups (1, 2) were with the addition of probiotics to the water during the experiment (Table 12, 13). The chickens were reared in a cage system; total of 18 cages with dimensions of 75x50 cm, divided by 9 cages into two compartments. The total number of chickens was 180 pieces (10 pieces in each cage) of one-day-old chickens with a density of 26.6 chickens per m². A total of 18 cages were divided between 3 groups (6 cages control group, 6 cages experimental group 1 and 6 cages experimental group 2). Watering as well as feeding the was carried out by the *ad libitum* system.

Heating in the 1st experiment and the following 10 experiments was provided by central heating. The air temperature was 33 °C on the first day and it was reduced by 2 °C every week until the final 21 °C. Air temperature and humidity were recorded daily by a data logger

(HIVUS Žilina, Slovak Republic). The light regime during the entire fattening period was continuous. Lighting of the breeding area was provided by 60 W light bulbs.

After 42 d of fattening, 30 pcs of chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Table 11: Composition and nutritional value of CFMs used in the experiment 1

Ingredients (%)	HYD-01	HYD-02	HYD-03
Wheat	22.40	25.00	27.20
Corn	40.00	37.50	40.00
Soy extruded flour (46 % NSs)	22.50	16.00	22.00
Rapeseed extruded flour	–	5.00	–
Meat bone meal	6.00	6.50	4.00
Danish fish meal	4.00	2.70	–
Rapeseed oil	1.10	3.00	3.00
Calcium carbonate	0.40	0.40	0.70
Premix Lysine 20%	1.60	1.80	0.55
Premix Methionine 20%	–	0.10	0.40
Sodium choride	–	–	0.15
Premix VM*	2.00	2.00	2.00
Nutritional value per 1 kg of CFMs (g.kg⁻¹)			
NSs (g)	220	200	180
Metabolized energy (MJ)	12.60	12.60	12.60
Lysine (g)	11.80	10.50	9.10
Met + Cys (g)	9.20	8.70	7.60
Ca (g)	10.00	8.00	7.50
Non-phytate P (g)	5.20	4.00	3.50

Note: NSs – nitrogen substances; *active substances per kilogram of vitamin – mineral premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; cobalamin 8.0 mg; choline 100 000 mg; betaine 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

Characteristic and composition of probiotic preparation

The probiotic preparation for chickens contained a specially selected strain of the genus *Lactobacillus* and was intended for the prevention and suppression of diarrheal diseases in poultry (mainly caused by *Escherichia coli* germs). The probiotic preparation was produced by company IPC Ltd., Košice.

Probiotic part: *Lactobacillus fermentum* 1.10⁹ CFU per 1 g of medium

Prebiotic part: Maltodextrin + oligofructose

Dosage of probiotic preparate during the experiment

Table 12: Experimental group 1 – drinking water with addition of selected probiotic preparate in liquid form (ml)

Week of age	Dosage of drinking water	Dosage of probiotics
1. week	2,500	6.6
2. week	3,5 10	6.6
3. week	4,6 10	3.7
4. week	6,7 10	3.7
5. week	8,6 10	3.7
6. week	10,6 10	3.7

Table 13: Experimental group 2 – drinking water with addition of selected probiotic preparate in liquid form (ml)

Week of age	Dosage of drinking water	Dosage of probiotics
1. week	2,500	3.3
2. week	3,5 10	3.3
3. week	4,6 10	3.3
4. week	6,7 10	3.3
5. week	8,6 10	3.3
6. week	10,6 10	3.3

3.2.2 2nd Experiment

The second experiment was carried out at the poultry testing station of the Department of Poultry and Small Livestock, at the Faculty of Agrobiolgy and Food Resources of the SUA in Nitra, on 180 broiler chickens of the Ross 308 hybrid combination. In the experiment, 3 groups of animals were created, control (C), experimental 1 (E1), experimental 2 (E2), in each 60 broiler chickens were included. Fattening of broiler chickens in both experiments lasted 42 days. Chickens were fed with starter CFM HYD-01 (loose form) until the 21st day of age and from the 22nd to the 42nd day of fattening CFM HYD-02 (granulated form) (Table 14). A probiotic preparation based on *Enterococcus faecium* (2.10^{10} CFU.g⁻¹) was added to the drinking water in the amount of 5.04 ml until the 2nd week of fattening and 2.10 ml until the

end of fattening (42 days) in the E1 group and in E2 group was added 10.08 ml until the 2nd week of fattening and 4.20 ml until the end of fattening (42 days). The chickens were reared in a cage system; total of 18 cages with dimensions of 75 x 50 cm, divided into 6 cages in 3 compartments; total number of 180 (10 chickens in each cage) one-day-old chickens Ross 308 with a density of 26.6 chickens per m². A total of 18 cages were divided between 3 groups (6 cages control group, 6 cages E1 and 6 cages E2 group). Watering as well as feeding was carried out by the *ad libitum* system.

After 42 d of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Table 14: Composition and nutritional value of CFMs used in the experiment 2

Ingredients (%)	HYD-01 – starter	HYD-02 – grower
Wheat	15.50	16.00
Corn	45.00	48.375
Soy extruded meal (47 %NSs)	36.00	32.00
Monocalcium phosphate	1.00	1.40
Premix 0,5 %	0.040	0.045
Premix VM*	0.055	0.065
Betafin S1	0.020	0.015
Calcium carbonate	1.684	1.350
Sodium chloride	0.400	0.300
Premix Lysine 99 %	0.150	0.250
Premix Methionine 99 %	0.150	0.200
Nutritional value per 1 kg of CFMs (g.kg⁻¹)		
NSs (g)	222.18	208.25
Metabolized energy (MJ)	11.907	12.065
Lys (g)	13.10	12.80
Met + Cys (g)	8.414	8.539
Ca (g)	9.09	8.41
Non-phytate P (g)	3.09	3.74

Note: NSs – nitrogen substances; *active substances per kilogram of vitamin - mineral premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; cobalamin 8.0 mg; choline 100 000 mg; betaine 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

Characteristic and composition of probiotic preparation

The probiotic preparation for chickens contained a specially selected strain of the genus *Enterococcus faecium*. The probiotic preparation was produced by IPC Ltd., Košice.

Probiotic part: *Enterococcus faecium* 2.10¹⁰ CFU.g⁻¹ of medium

Prebiotic part: Dextrose

3.2.3 3rd Experiment

The third experiment was carried out at the poultry testing station of the Department of Poultry and Small Livestock, at the Faculty of Agrobiological and Food Resources of the SUA in Nitra, on 180 Ross 308 broiler chickens. In the experiment, 3 groups of animals were created, control (C), experimental 1 (E1), experimental 2 (E2), where 60 chickens were included in each group. Own fattening of broiler chickens lasted 42 days. Fattening of broiler chickens in both experiments lasted 42 days. Chickens were fed with starter CFM HYD-01 (loose form) until the 21st day of age and from the 22nd to the 42nd day of fattening CFM HYD-02 (granulated form) (Table 15). The experimental groups were given a probiotic preparation based on strains of *Lactobacillus* sp., *Streptococcus thermophilus* and *Enterococcus faecium* in a concentration of min. $2 \cdot 10^9$ CFU.1 g⁻¹ of medium to water in the amount of 6 g during the entire fattening period (E1 group) and 3 g during the entire fattening period (E2 group). The chickens were reared in a cage system; total of 18 cages with dimensions of 75 x 50 cm, divided into 6 cages in 3 compartments; total number of 180 (10 chickens in each cage) one-day-old chickens Ross 308 with a density of 26.6 chickens per m⁻². A total of 18 cages were divided between 3 groups (6 cages control group, 6 cages experimental group 1 and 6 cages experimental group 2). Watering as well as feeding was carried out by the *ad libitum* system.

After 42 d of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Table 15: Composition and nutritional value of CFMs used in the experiment 3

Ingredients (%)	HYD-01 – starter	HYD-02 – grower
Wheat	15.50	16.00
Corn	45.00	48.375
Soy extruded meal (47 %NSs)	36.00	32.00
Monocalcium phosphate	1.00	1.40
Premix 0,5 %	0.040	0.045
Premix VM*	0.055	0.065
Betafin S1	0.020	0.015
Calcium carbonate	1.684	1.350
Sodium chloride	0.400	0.300
Premix Lysine 99 %	0.150	0.250
Premix Methionine 99 %	0.150	0.200
Nutritional value per 1 kg of CFMs (g.kg⁻¹)		
NSs (g)	222.18	208.25
Metabolized energy (MJ)	11.907	12.065
Lys (g)	13.10	12.80
Met + Cys (g)	8.414	8.539
Ca (g)	9.09	8.41
Non-phytate P (g)	3.09	3.74

Note: NSs – nitrogen substances; *active substances per kilogram of vitamin - mineral premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; cobalamin 8.0 mg; choline 100 000 mg; betaine 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

Characteristic and composition of probiotic preparation

Lactina is a probiotic preparation with a multi-strain composition. It contains strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* LAT187, *Lactobacillus acidophilus* LAT180, *Lactobacillus helveticus* LAT179, *Lactobacillus delbrueckii* ssp. *lactis* LAT182, *Streptococcus thermophilus* LAT205 and *Enterococcus faecium* E-253 in a concentration of min. $2 \cdot 10^9$ CFU in 1 g of medium. The probiotic preparation has antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Listeria*, *Proteus mirabilis* and other facultative pathogenic bacteria. It improves the balance in the digestive tract of animals and has a beneficial effect on the immune system. The preparation is produced by the Austrian company PROCHEMA

Handelsgesellschaft m.b.H., Wien and its supplier in the Slovak Republic is AGROkonzult, ltd., Branovo.

3.2.4 4th Experiment

The fourth experiment was carried out in the poultry testing station of the Department of Poultry and Small Livestock, at the Faculty of Agrobiological and Food Resources of the SUA in Nitra on broiler chickens of the hybrid combination Ross 308. 180 one-day-old chickens were included in the experiment, and subsequently 2 groups of animals were created: control (C) and experimental (E) 90 chickens each. The fattening period lasted 42 days. Chickens were fed with the starter CFM HYD-01 (loose structure) from the 1st to the 21st day of age and from the 22nd to the 42nd day with the growth CFM HYD-02 (loose structure) in both monitored groups (Table 16). The CFMs HYD-01 and HYD-02 were produced without antibiotic preparations and coccidiostats. The average nutritional value of the feed mixtures administered during the experiment was the same in both groups, but in the experimental group propolis extract was additionally added to the used feed mixtures at a dose of 0.2 g.kg⁻¹. The chickens were fed and watered by the *ad libitum* system. The chickens were housed on deep bedding.

Propolis extract was prepared from ground propolis in the conditions of the 80% ethanol in the 500 cm³ flasks, according to **Krell (1996)**. The extraction was accomplished in a water bath at 80 °C under reflux cooler for one hour. After that, the extracts were cooled and centrifuged. The obtained supernatants were evaporated in a rotary vacuum evaporator at bath temperature 40 – 50 °C, and then weighed. Finally, the residues in an appropriate amount (depending on addition of supplement per kg of feed) was dissolved in ethanol and applied to the feed mixture through a bearing medium.

After 42 d of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Table 16: Composition and nutritional value of CFMs used in the experiment 4

Ingredients (%)	HYD-01 – Starter	HYD-02 – Grower
Wheat	34.00	37.00
Corn	33.92	37.52
Soy extruded meal (48 % NL¹)	23.00	18.00
Fish meal (71 % NSs)	5.00	3.00
Dried blood	-	1.00
Calcium carbonate	1.00	0.95
Monocalcium phosphate	0.80	0.70
Sodium chloride	0.10	0.10
Sodium bicarbonate	0.15	0.20
Lysine HCL	0.13	0.08
Methionine	0.18	0.20
Clinacox 0,5 %²	0.02	-
Palm kernel fat Bergafat	1.20	0.70
Sacox 12 %³	-	0.05
Premix VM 0,5 %⁴	0.50	0.50
Nutritional value per 1 kg of CFMs (g.kg⁻¹)		
NSs	212.40	191.62
Fiber	30.51	29.68
Ash	27.01	20.90
Ca	8.23	7.18
P	6.56	5.87
Mg	1.77	1.71
Linoleic acid	13.53	14.06
Metabolized energy (MJ)	12.07	12.16

¹ NSs – nitrogen substances; ² active substance diclazuril; ³ active substance sodium salomycinate, ⁴ active substances in 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 acid 3 000 mg; riboflavin 1 800 mg; pyridoxin 1 200 mg; thiamin 600 mg; menadione 800 mg; ascorbic acid; 50 000 mg; folic acid 400 mg; biotin 40 mg; vitamin B₁₂ 10 mg; choline 100 000 mg; betaine 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.

3.2.5 5th Experiment

The 5. experiment was carried out in test poultry station of Slovak Agricultural University in Nitra. In the experiment was included 180 one-day-old chickens of Ross 308 hybrid combinations, divided into 6 groups according to the added preparation with various amount of pollen and propolis. Two-phase fattening was used with CFMs of HYD-01 (1. – 21. day) and continued with HYD-02 (22. – 42. day) (Table 17).

Fattening lasted 42 days and the chickens were fed and watered *ad libitum*. CFMs were produced without antibiotics and growth stimulants. Propolis and pollen extracts were administered to experimental groups in feed mixtures HYD-01 and HYD-02 as follows: the addition of propolis 200 mg.kg⁻¹ CFM (E1), the addition of propolis 300 mg.kg⁻¹ CFM (E2), with the addition of propolis 400 mg.kg⁻¹ CFM (E3), with the addition of pollen 400 mg.kg⁻¹ CFM (E4) and with the addition of pollen 800 mg.kg⁻¹ CFM (E5). The control group was without the addition of propolis and pollen extracts.

The chickens were reared in a cage system; total of 18 cages with dimensions of 75 x 50 cm, divided by 3 cages into 6 compartments with a density of 26.6 chickens per m² (10 chickens in each cage). Cages were divided among 6 groups (3 cages control group, 3 cages experimental group E1, E2, E3, E4 and E5).

Propolis and pollen extract was prepared from their natural ground form in the conditions of the 80% ethanol in the 500 cm³ flasks, according to **Krell (1996)**. The extraction was accomplished in a water bath at 80 °C under reflux cooler for one hour. After that, the extracts were cooled and centrifuged. The obtained supernatants were evaporated in a rotary vacuum evaporator at bath temperature 40 – 50 °C, and then weighed. Finally, the residues in an appropriate amount (depending on addition of supplement per kg of feed) was dissolved in ethanol and applied to the feed mixture through a bearing medium.

After 42 d of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Table 17: Composition and nutritional value of CFMs used in the experiment 5

Ingredients (%)	HYD-01 – Starter	HYD-02 – Grower
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48 % N)	21.30	18.70
Fish meal (71 % N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysine	0.05	0.07
Methionine	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5 %*	0.50	0.50
Nutritional value per 1 kg of CFMs (g.kg⁻¹)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
ME (MJ.kg⁻¹)	12.02	12.03

Notes: *active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 50000 mg; folic acid 400 mg; biotin 40 mg; vitamin B12 10.0 mg; choline 100000 mg; betaine 50000 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

3.2.6 6th Experiment

The 6. experiment has been implemented in the test poultry station of Slovak University of Agriculture in Nitra. The tested chicken was hybrid combination Ross 308. The chickens were bred in a cage conditions. Each cage was equipped with feed dispenser and water intake was ensured *ad libitum* through a self-feed pump. Fattening lasted 42 days. Each group was fed

by same starter CFM (loose structure) until to 21st day of their age and broiler fed by the grower from the 22nd to 42nd day of their age. The CFMs starter and grower had been produced without antibiotic preparations and coccidiostats and their composition is presented in Table 18. The experiment enrolled 360 one-day-old chicks, which were divided into 6 groups (n=60): control (C) and experiments (E1, E2, E3, E4 and E5). In the experimental groups was added natural bee pollen to CFMs in amount 500 mg.kg⁻¹ (E1), 1500 mg.kg⁻¹ (E2) and 2500 mg.kg⁻¹ (E3), 3500 mg.kg⁻¹ (E4) and 4500 mg.kg⁻¹ (E5).

Fattening trials with broiler chickens Ross 308 hybrid combinations were implemented in three store cages, technology from STL 1000/615 Salmela (Slovakia). Each cage has a size of 70x100 cm (0.70 m²).

After 42 days of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Table 18: Composition and nutritional value of CFMs used in the experiment 6

Ingredients (%)	HYD-01 – Starter	HYD-02 – Grower
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48 % N)	21.30	18.70
Fish meal (71 % N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysine	0.05	0.07
Methionine	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5 %*	0.50	0.50
Nutritional value per 1 kg of CFMs (g.kg⁻¹)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
ME (MJ.kg⁻¹)	12.02	12.03

Notes: *active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 50000 mg; folic acid 400 mg; biotin 40 mg; vitamin B12 10.0 mg; choline 100000 mg; betaine 50000 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

3.2.7 7th Experiment

The 7. experiment included 360 one-day-old broiler chickens (Ross 308) of mixed sex randomly divided into 6 groups (each containing 60 chickens). Chickens were given the feed supplements during the entire fattening period. The experiment was conducted in the test poultry station of Slovak University of Agriculture in Nitra. The experiment lasted for 42 days. The size of pen for one group of chickens (60 pcs) was 3.2 × 2.4 m. The broiler chickens were reared on breed litter (wood shavings). Over the entire fattening period, the chickens were provided with *ad libitum* access to feed (mash form) and drinking water. Diets were formulated to provide the nutrient requirements of broilers according to the recommended reference levels (**Bulletin of MARD SR, 2005**), and broilers received two phases feeding program, starter HYD-01 (1 – 21 d) and grower HYD-02 (22 – 42 d) diets. Ingredient and nutrient content of the basal diets is presented in Table 20. Feed mixtures were prepared by Biofeed, Inc. (Kolárovo, Slovak Republic). The feed mixtures both starter and grower were produced without any antibiotics and coccidiostats.

After 42 days of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Bee pollen and propolis extracts preparation

Bee pollen and propolis used in the experiment have originated from the Slovak Republic. The extracts were prepared from minced bee pollen and propolis in the conditions of the 80% ethanol in the 500 cm³ flasks, according to **Krell (1996)**. The extraction was accomplished in a water bath at 80 °C under reflux cooler for one hour. After that, the extracts were cooled and centrifuged. The obtained supernatants were evaporated in a rotary vacuum evaporator at bath temperature 40 – 50 °C, and then weighed. Finally, the residues in an appropriate amount (depending on addition of supplement per kg of feed) was dissolved in ethanol and applied to the feed mixture through a bearing medium.

Each experiment employed a randomized design, and dietary treatments were as follows:

- 1) control group: basal diet without supplementation (C);
- 2) 1st experimental group: basal diet plus 400 mg bee pollen extract per 1 kg of feed mixture (E1);

- 3) 2nd experimental group: basal diet plus 400 mg propolis extract per 1 kg of feed mixture (E2);
- 4) 3rd experimental group: basal diet plus 3.3 g probiotic (please refer to Table 19) added daily to drinking water (E3);
- 5) 4th experimental group: basal diet plus 400 mg bee pollen extract per 1 kg of feed mixture and 3.3 g probiotic added daily to drinking water (E4);
- 6) 5th experimental group: basal diet plus 400 mg propolis extract per 1 kg of feed mixture and 3.3 g probiotic added daily to drinking water (E5).

Table 19: Probiotic dosing pattern via drinking water

Week of age	Amount of water per day for 60 chickens (l)	Amount of probiotic per day for 60 chickens (g)
1.	2.49	3.3
2.	3.51	3.3
3.	4.59	3.3
4.	6.69	3.3
5.	8.61	3.3
6.	10.59	3.3

Table 20: Composition and nutritional value of CFMs used in the experiment 7

Ingredients (%)	HYD-01 – Starter	HYD-02 – Grower
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48 % N)	21.30	18.70
Fish meal (71 % N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysine	0.05	0.07
Methionine	0.15	0.22

Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5 %*	0.50	0.50
Nutritional value per 1 kg of CFMs (g.kg⁻¹)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
ME (MJ.kg⁻¹)	12.02	12.03

Notes: *active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 50000 mg; folic acid 400 mg; biotin 40 mg; vitamin B12 10.0 mg; choline 100000 mg; betaine 50000 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

3.2.8 8th Experiment

The 8. experiment included 360 one-day-old broiler chicks (Ross 308) of mixed sex randomly divided into 6 groups (each containing 60 chickens). The experiment was conducted in the test poultry station of Slovak University of Agriculture (SUA) in Nitra and lasted for 42 days. The size of pen for one group of chickens (60 pcs) was 3.2 × 2.4 m. The broiler chickens were reared on breed litter (wood shavings). Over the entire fattening period, the chickens were provided with *ad libitum* access to feed (mash form) and drinking water. Diets were formulated to provide the nutrient requirements of broilers according to the recommended reference levels (**Bulletin of MARD SR, 2005**), and broilers received two phases feeding program, starter HYD-01 (1 – 21 d) and grower HYD-02 (22 – 42 d) diets. Ingredient and nutrient content of the basal diets is presented in Table 22 Chyba! Nenašiel sa žiaden zdroj odkazov.. Feed mixtures were prepared by Biofeed, Inc. (Kolárovo, Slovak Republic). The feed mixtures both starter and grower were produced without any antibiotics and coccidiostats.

After 42 d of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Characterization of probiotic preparation applied in experiment

In the experiment, two-component probiotic preparation containing probiotic microorganism *Lactobacillus fermentum* (1×10^9 CFU in 1 g of bearing medium) and a potentiating component (maltodextrin and oligofructose) was used.

This probiotic preparation is used in poultry to improve their immunity and metabolism, stimulate the growth, as well as to prevent and suppress the diarrhoea disease (caused by pathogen *E. coli*). It can inhibit pathogenic microorganisms and is suitable for farming conditions. The economic effect of addition of probiotic preparation is reflected in reduced mortality, intestinal diseases, and thus the costs of therapy. The probiotic preparation was supplied by IPC Ltd. (Košice, Slovak Republic).

Bee pollen and propolis extracts preparation

Bee pollen and propolis used in the experiment have originated from the Slovak Republic. The extracts were prepared from minced bee pollen and propolis in the conditions of the 80% ethanol in the 500 cm³ flasks, according to **Krell (1996)**. The extraction was accomplished in a water bath at 80 °C under reflux cooler for one hour. After that, the extracts were cooled and centrifuged. The obtained supernatants were evaporated in a rotary vacuum evaporator at bath temperature 40 – 50 °C, and then weighed. Finally, the residues in an appropriate amount (depending on addition of supplement per kg of feed) was dissolved in ethanol and applied to the feed mixture through a bearing medium.

Each experiment employed a randomized design, and dietary treatments were as follows:

- 1) control group: basal diet without supplementation (C);
- 2) 1st experimental group: basal diet plus 6 g probiotic added daily to drinking water (E1);
- 3) 2nd experimental group: basal diet plus 12 g probiotic added daily to drinking water (E2);
- 4) 3rd experimental group: basal diet plus 500 mg bee pollen extract per 1 kg of feed mixture (E3);
- 5) 4th experimental group: basal diet plus 500 mg propolis extract per 1 kg of feed mixture (E4);
- 6) 5th experimental group: basal diet plus 600 mg propolis extract per 1 kg of feed mixture (E5).

Table 21: Probiotic dosing pattern via drinking water

Week of age	Amount of water per day for 60 chickens (l)	Amount of probiotic per day for 60 chickens (g) E1/E2
1.	2.49	6.0/12.0
2.	3.51	6.0/12.0
3.	4.59	6.0/12.0
4.	6.69	6.0/12.0
5.	8.61	6.0/12.0
6.	10.59	6.0/12.0

Table 22: Composition and nutritional value of CFMs used in the experiment 8

Ingredients (%)	HYD-01 – Starter	HYD-02 – Grower
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48 % N)	21.30	18.70
Fish meal (71 % N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysine	0.05	0.07
Methionine	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5 %*	0.50	0.50
Nutritional value per 1 kg of CFMs (g.kg⁻¹)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
ME (MJ.kg ⁻¹)	12.02	12.03

Notes: *active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 50000 mg; folic acid 400 mg; biotin 40 mg; vitamin B12 10.0 mg; choline 100000 mg; betaine 50000 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

3.2.9 9th Experiment

We conducted the 9th experiment with the broiler chickens of the hybrid combination Ross 308 in the Test poultry station of Slovak University of Agriculture in Nitra. The experiment was carried out with a breeding system on deep litter (shavings). 600 one-day-old chickens of the commercial hybrid Ross 308 were included in the experiment. Subsequently, a control group (without the application of feed supplements in CFMs) and 5 experimental groups (different dosages of feed supplements in feed mixtures and in water – Table 24). Each group included 100 pcs of broiler chickens. The fattening of the chickens lasted 42 days.

Feed was given using tube feeders. The feed mixtures used in the experiments were mixed and prepared in the company De Heuse, JSC. with headquarters in Marefy (Czech Republic) according to the requirements of the **Bulletin of MARD (2004) (Table xy)**. The feed was given manually daily at regular intervals with the same starter CFM HYD-01 from the 1st to the 10th day of age with the coccidiostat Maxiban; CFM HYD-02 – growth feed mixture I., fed from the 11th to the 20th day with the coccidiostat Salocin; CFM HYD-03 – growth feed mixture II, fed from the 21st to the 28th day with the coccidiostat Salocin, and from the 29th to the 42nd day of age the final CFM HYD-04 was given to the broiler chickens. All CFMs were in loose form and their composition is presented in the Table 23. Water and feed were given *ad libitum* system using bucket drinkers.

After 42 d of fattening, 30 pcs chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the slaughterhouse of Institute of Food Sciences (SUA, Nitra).

Table 23: Composition and nutritional value of CFMs used in the experiment 9

Ingredients (%)	Starter CFM (1. – 10. day)	Grow CFM I. (11. – 20. day)	Grow CFM II. (21. – 35. day)	Final CFM (36. – 42. day)
Corn	46.33	48.50	50.05	50,91
Wheat	14.00	15.00	15.00	15,00
Soy extruded meal (45% NSs)	30.00	26.60	28.00	26,70
Fishmeal (72 % NSs)	2.50	2.00	-	-

Dried blood	2.00	2.00	-	-
Soy oil	1.00	1.80	2.80	3.00
Monocalcium phosphate	1.60	1.25	1.30	1.48
Calcium carbonate	1.37	1.55	1.50	1.56
Fodder salt	0.20	0.30	0.35	0.35
L-Lysine	0.27	0.15	0.15	0.16
DL-Methionine	0.27	0.18	0.17	0.20
L-Threonine	0.09	0.10	0.08	0.07
¹Vitamin premix	0.05	0.04	0.04	0.03
²Mineral premix	0.04	0.04	0.04	0.04
Enzyme phytase	0.015	0.015	0.015	0.015
Wheat flour	0.215	0.12	0.10	0.135
Maxiban (Narasin+Nicarbasin)	0.05	-	-	-
Sacox (sodium salicynemycitate)	-	0.055	0.055	-
Nutritional value per 1 kg of CFMs (g.kg⁻¹)				
NSs	220.00	207.00	197.00	188.00
Fiber	20.00	24.00	28.00	29.00
Lysine	14.00	12.50	12.50	11.50
Methionine	6.00	5.20	5.20	5.00
Ca	9.00	8.50	8.50	8.50
Non-phytate P	4.20	4.00	4.00	4.00
Na	1.60	1.60	1.60	1.60
Metabolized energy (MJ.kg⁻¹)	12.30	12.75	13.15	13.15

Notes: NSs – nitrogen substances; ¹ active substances in the vitamin premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D 3 800 000 IU; niacin 12 000 mg; pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxin 1 200 mg; thiamin 600 mg; menadione 800 mg; ascorbic acid 50 000 mg; folic acid 400 mg; biotin 40 mg; vitamin B₁₂ 10 mg; choline 100 000 mg; betaine 50 000 mg; ³active substances in the 1 kg of mineral premix: 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.

Description of feed phyto-additives used in the experiment

Agolin Poultry:

- is a mixture of high-quality plant active substances from lavender, clove and black pepper designed to optimize the animal's food intake and improve its digestive functions.

Agolin Tannin Plus:

- is a mixture of plant active substances from chestnut extract and citrus fruits with hydrolysable tannins for improving the activity of the digestive system of animals, while maintaining feed intake and efficiency.

Biostrong 510:

- is a mixture of plant essential oils (thyme and star anise) and saponins from plants with organic acids that improve the palatability of feed and the microclimate of the breeding environment.

FortiBac:

- is a glycerides mixture of butyric, propionic, caprylic, capric acids and free glycerol bound to silicon dioxide. The product is intended for the prevention of bacterial diseases of the digestive tract, for the rapid regeneration of the intestinal mucosa, and at the same time as an excellent source of ready energy for young animals that are not yet able to digest fats (triglycerides) due to insufficiently developed pancreatic lipase and bile production.

Agolin Acid:

- is a mixture of high-quality plant essential oils from bergamot, clove, thyme, black pepper and preservatives represented by fumaric and citric acid. The preparation is designed for optimal feed intake and ensuring optimal digestive functions for poultry.

Biocitro:

- is a mixture of ascorbic acid, bioflavonoids, sugars, polyphenols and fatty acids from citrus fruits, which has a positive effect on the physiological functions in the animal's organism, helps to suppress pathogenic microorganisms in the intestinal microflora, thereby having a beneficial effect on the function of the digestive tract and overall improving the health of the animal.

Table 24: Phytoadditives dosage in the experiment

Group	Phytoadditive / dosage
Control	-
1. experimental	Agolin Poultry (0,1 g.kg ⁻¹ CFM)
2. experimental	Agolin Tannin Plus (0,5 g.kg ⁻¹ CFM)
3. experimental	Biostrong 510+FortiBac (1,0 g.kg ⁻¹ CFM)
4. experimental	Agolin acid (1,0 g.kg ⁻¹ CFM)
5. experimental	Biocitro (1,0 ml.l ⁻¹ water)

3.2.10 10th Experiment

The 10. experiment included 400 one-day-old broiler chicks (Ross 308) of mixed sex randomly divided into 8 groups (each containing 50 chickens). Chickens were given the feed supplements during the entire fattening period. The experiment was conducted in the test poultry station of Slovak University of Agriculture in Nitra. The experiment lasted for 42 days.

Diets were prepared according to the recommended reference levels for broiler chickens (**Bulletin of MARD SR, 2005**). Broilers Ross 308 were feed with starter HYD-01 (1 – 21 d) and grower HYD-02 (22 – 42 d) diets. The starter and grower feed mixtures were produced without any antibiotics and coccidiostats and were prepared by Biofeed, Inc. (Kolárovo, Slovak Republic) (Table 25).

The experimental groups were set up as follows: the control group (C) involved the basal diet without supplementation; the experimental groups of chickens E1, E2 and E3 were fed with basal diet plus white grape seed polyphenols 450, 600 and 700 mg.100 kg⁻¹ of feed mixture, respectively. The experimental groups were set up as follows: the control group (C) involved the basal diet without supplementation; the experimental groups of chickens E1, E2 and E3 were fed with basal diet plus red grape pomace (variety Alibernet) 1, 2 and 3%.100 kg⁻¹ of CFM, respectively; experimental groups E4 and E5 were fed with a CFM plus flax pomace 2 and 4%.100 kg⁻¹ of CFM, respectively and experimental groups E6 and E7 were fed with a complete FM plus pumpkin pomace 2 and 4%.100 kg⁻¹ of feed mixture, respectively. Chickens were fed by *ad libitum* system.

At 42 days of age, 30 chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the experimental slaughterhouse of the Institute of Food Sciences (SUA, Nitra).

Table 25: Composition and nutritional value of CFMs used in the experiment 10

Ingredients (%)	Starter (HYD-01) (1st – 21st day of age)	Grower (HYD-02) (22nd – 42th day of age)
Wheat	34.50	30.00
Maize	28.00	39.00
Soybean meal (48% N)	31.00	26.00
Fodder lime	0.65	0.60
Calcium formate	0.80	0.80
Monocalcium phosphate	0.90	0.55
Fodder salt	0.20	0.20
Sodium bicarbonate	0.20	0.20
Lysine	0.10	0.05
Methionine	0.15	0.15
Soybean oil	3.00	1.95
Premix VMZ 0.5%*	0.50	0.50
Nutrient content (g.kg⁻¹)		
Linoleic acid	27.82	24.04
Fibre	28.71	27.84
Crude protein	209.68	189.60
Ash	45.45	39.59
Ca	8.12	7.27
P	6.04	5.13
Na	1.61	1.58
ME_N (MJ.kg⁻¹)	11.92	11.92

*active substances per kilogram of premix: vitamin A 2,500,000 IU; vitamin E 50,000 mg; vitamin D3 800,000 IU; niacin 12,000 mg; D-pantothenic acid 3,000 mg; riboflavin 1,800 mg; pyridoxine 1,200 mg; thiamine 600 mg; methadione 800 mg; ascorbic acid 50,000 mg; folic acid 400 mg; biotin 40 mg; cobalamin 10.0 mg; choline 100,000 mg; betaine 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.

3.2.11 11th Experiment

The last observed experiment included 400 one-day-old broiler chicks (Ross 308) of mixed sex randomly divided into 8 groups (each containing 50 chickens). Chickens were given the feed supplements during the entire fattening period. The experiment was conducted in the test poultry station of Slovak University of Agriculture (SUA) in Nitra. The experiment lasted for 42 days.

Diets were prepared according to the recommended reference levels for broiler chickens (**Bulletin of MARD SR, 2005**). Broilers Ross 308 were feed with starter HYD-01 (1 – 21 d) and grower HYD-02 (22 – 42 d) diets. The starter and grower feed mixtures were produced without any antibiotics and coccidiostats and were prepared by Biofeed, Inc. (Kolárovo, Slovak Republic) (Table 26). The experimental groups were set up as follows: the control group (C) involved the basal diet without supplementation; the experimental groups of chickens E1, E2 and E3 were fed with basal diet plus white grape seed polyphenols 450, 600 and 700 mg.100 kg⁻¹ of feed mixture, respectively. Chickens were fed by *ad libitum* system.

At 42 days of age, 30 chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the experimental slaughterhouse of the Institute of Food Sciences (SUA, Nitra).

Table 26: Composition and nutritional value of CFMs used in the experiment 11

Ingredients (%)	Starter (HYD-01) (1 st – 21 st day of age)	Grower (HYD-02) (22 nd – 42 th day of age)
Wheat	34.50	30.00
Maize	28.00	39.00
Soybean meal (48% N)	31.00	26.00
Fodder lime	0.65	0.60
Calcium formate	0.80	0.80
Monocalcium phosphate	0.90	0.55
Fodder salt	0.20	0.20
Sodium bicarbonate	0.20	0.20
Lysine	0.10	0.05
Methionine	0.15	0.15
Soybean oil	3.00	1.95
Premix VMZ 0.5%*	0.50	0.50

Nutrient content (g.kg⁻¹)		
Linoleic acid	27.82	24.04
Fibre	28.71	27.84
Crude protein	209.68	189.60
Ash	45.45	39.59
Ca	8.12	7.27
P	6.04	5.13
Na	1.61	1.58
ME_N (MJ.kg⁻¹)	11.92	11.92

*active substances per kilogram of premix: vitamin A 2,500,000 IU; vitamin E 50,000 mg; vitamin D3 800,000 IU; niacin 12,000 mg; D-pantothenic acid 3,000 mg; riboflavin 1,800 mg; pyridoxine 1,200 mg; thiamine 600 mg; methadone 800 mg; ascorbic acid 50,000 mg; folic acid 400 mg; biotin 40 mg; cobalamin 10.0 mg; choline 100,000 mg; betaine 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.

3.3 Monitored indicators of the experiment

Indicators of meat performance:

- Carcass weight (CW),
- Abdominal fat weight (g),
- Abdominal fat proportion of carcass (%).

3.4 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 different feed supplements as an addition to complete feed mixtures and water in the nutrition of chickens of the hybrid combination Ross 308 on the formation of abdominal fat in carcass was studied. Results are presented in the Tables 27 – 37.

Table 27: Experiment 1 – Effect of probiotic preparate containing *Lactobacillus fermentum* on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	P-value
CW (g)	1468.25±54.26	1540.00±111.03	1539.88±114.47	0.257
AF (g)	25.35±3.87 ^b	30.73±2.86 ^a	29.28±3.07 ^a	0.011
AF from CW (%)	1.73±0.29	2.01±0.26	1.91±0.23	0.129

Notes: C – control group; E1, E2 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 28: Experiment 2 – Effect of probiotic preparate containing *Enterococcus faecium* on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	P-value
CW (g)	1108.75±38.43 ^b	1255.50±73.61 ^a	1286.63±112.73 ^a	0.001
AF (g)	15.38±4.76 ^b	20.88±3.07 ^a	21.20±2.97 ^a	0.007
AF from CW (%)	1.38±0.41	1.67±0.27	1.65±0.21	0.145

Notes: C – control group; E1, E2 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 29: Experiment 3 – Effect of probiotic preparate containing *Lactobacillus spp.*, *Streptococcus thermophilus* and *Enterococcus faecium* on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	P-value
CW (g)	1356.75±37.53 ^b	1354.38±85.08 ^b	1449.13±73.92 ^a	0.017

AF (g)	20.24±4.81	21.71±6.19	23.06±6.82	0.648
AF from CW (%)	1.49±0.33	1.60±0.44	1.59±0.43	0.842

Notes: C – control group; E1, E2 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 30: Experiment 4 – Effect of propolis extract on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	P-value
CW (g)	1402.00±119.34	1434.75±118.65	0.591
AF (g)	23.11±4.98	21.86±3.58	0.575
AF from CW (%)	1.65±0.34	1.52±0.21	0.392

Notes: C – control group; E1– experimental group; CW – carcass weight; AF – abdominal fat

Table 31: Experiment 5 – Effect of bee pollen and propolis on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	E3	E4	E5	P-value
CW (g)	1584.63 ±94.52	1629.88 ±63.38	1630.88 ±99.37	1663.38 ±93.52	1569.75 ±76.00	1644.13 ±78.85	0.236
AF (g)	27.21 ±4.59	27.16 ±7.72	28.52 ±4.37	29.22 ±4.91	25.59 ±3.98	26.49 ±4.02	0.747
AF from CW (%)	1.72 ±0.28	1.66 ±0.44	1.76 ±0.35	1.75 ±0.24	1.63 ±0.27	1.61 ±0.23	0.886

Notes: C – control group; E1, E2, E3, E4, E5 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 32: Experiment 6 – Effect of bee pollen on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	E3	E4	E5	P-value
CW (g)	1644.38	1629.25	1607.13	1555.75	1589.63	1461.25	0.007

	±84.24 ^a	±110.57 ^a	±138.75 ^a	±94.82 ^{ab}	±73.43 ^a	±70.91 ^b	
AF (g)	35.82 ±3.97 ^a	30.12 ±5.10 ^{abc}	33.42 ±6.31 ^{abc}	35.09 ±6.94 ^{ab}	29.55 ±4.75 ^{bc}	28.51 ±4.01 ^c	0.032
AF from CW (%)	2.18 ±0.21	1.86 ±0.35	2.07 ±0.28	2.25 ±0.41	1.87 ±0.34	1.95 ±0.28	0.083

Notes: C – control group; E1, E2, E3, E4, E5 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 33: Experiment 7 – Effect of bee pollen, propolis and probiotic preparation on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	E3	E4	E5	P-value
CW (g)	1645.00 ±66.83	1707.25 ±85.09	1650.13 ±96.09	1661.50 ±97.37	1699.88 ±80.68	1674.13 ±146.19	0.738
AF (g)	22.42 ±2.77	22.82 ±3.13	21.65 ±4.19	23.68 ±3.73	24.94 ±4.13	23.78 ±4.05	0.567
AF from CW (%)	1.37 ±0.19	1.34 ±0.18	1.32 ±0.29	1.42 ±0.20	1.48 ±0.29	1.43 ±0.26	0.767

Notes: C – control group; E1, E2, E3, E4, E5 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 34: Experiment 8 – Effect of bee pollen, propolis and probiotic preparation on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	E3	E4	E5	P-value
CW (g)	1327.75 ±83.22	1387.75 ±84.52	1356.63 ±102.24	1411.88 ±108.19	1410.13 ±100.73	1449.88 ±76.00	0.146
AF (g)	16.90 ±3.44	18.40 ±3.76	15.82 ±3.45	19.46 ±3.20	19.76 ±3.04	19.18 ±3.04	0.138
AF from CW (%)	1.28 ±0.26	1.32 ±0.22	1.17 ±0.25	1.39 ±0.26	1.40 ±0.20	1.33 ±0.22	0.408

Notes: C – control group; E1, E2, E3, E4, E5 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 35: Experiment 9 – Effect of phytoadditives on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	E3	E4	E5	P-value
CW (g)	1308.80 ±63.79	1390.50 ±60.31	1317.33 ±69.30	1341.83 ±73.57	1367.00 ±92.65	1350.83 ±24.57	0.315
AF (g)	14.74 ±4.94	12.96 ±4.56	9.84 ±2.64	11.53 ±6.91	9.87 ±4.19	11.61 ±3.79	0.454
AF from CW (%)	1.12 ±0.37	0.94 ±0.35	0.75 ±0.23	0.87 ±0.54	0.71 ±0.27	0.86 ±0.27	0.414

Notes: C – control group; E1, E2, E3, E4, E5 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 36: Experiment 10 – Effect of grape, flax and pumpkin pomace on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	E3	E4	E5	E6	E7	P-value
CW (g)	1446.38 ±89.09	1417.50 ±109.30	1484.25 ±90.35	1502.25 ±53.48	1385.75 ±180.28	1453.88 ±102.38	1357.38 ±102.53	1455.75 ±67.16	0.127
AF (g)	10.81 ±3.84 ^{ab}	10.53 ±2.48 ^{ab}	12.65 ±4.58 ^a	12.58 ±3.07 ^a	8.98 ±2.15 ^b	9.55 ±2.72 ^{ab}	7.78 ±1.53 ^b	8.61 ±1.75 ^b	0.010
AF from CW (%)	0.74 ±0.25 ^{ab}	0.75 ±0.15 ^{ab}	0.80 ±0.26 ^{ab}	0.84 ±0.19 ^a	0.65 ±0.15 ^{ab}	0.65 ±0.16 ^{ab}	0.57 ±0.10 ^b	0.59 ±0.10 ^b	0.019

Notes: C – control group; E1, E2, E3, E4, E5, E6, E7 – experimental groups; CW – carcass weight; AF – abdominal fat

Table 37: Experiment 11 – Effect of grape seeds polyphenols on formation of abdominal fat in the carcass of Ross 308 broiler chickens

Group	C	E1	E2	E3	P-value
CW (g)	1996.55 ±214.04	1935.58 ±277.22	1985.30 ±344.48	1998.69 ±190.91	0.959

AF (g)	23.99±3.95 ^{ab}	19.77±5.32 ^b	20.88±4.03 ^b	27.22±7.12 ^a	0.037
AF from CW (%)	1.20±0.18 ^{ab}	1.01±0.19 ^b	1.07±0.26 ^b	1.36±0.31 ^a	0.039

Notes: C – control group; E1, E2, E3 – experimental groups; CW – carcass weight; AF – abdominal fat

Based on the results achieved from 11 experiments after the application of various feed supplements in the nutrition of Ross 308 chickens through the feed mixture, or through water, we can state that the weight of abdominal fat was from 7.78 g (experiment 10, E6 group – addition of pumpkin pomace – 2%.100 kg FM⁻¹) to 35.09 g (experiment 6, E3 group – addition of natural bee pollen – 2500 mg.kg FM⁻¹).

The highest abdominal fat mass was achieved in experiment 6, in the control group (35.82 g).

Significant differences ($P \leq 0.05$) in the weight of abdominal fat in individual experiments were achieved in experiment 1 with the addition of a probiotic preparation based on *Lactobacillus fermentum*, where the experimental groups had higher values of 5.38 g (E1) and 3.93 g (E2). Similarly, higher abdominal fat mass values were achieved in experiment 2 after the application of the probiotic preparation based on *Enterococcus faecium*, where the values were higher by 5.50 g in group E1 and 5.82 g in group E2 compared to the control group, where the value was 15.38 g.

The opposite tendency, i.e., a significant decrease in abdominal fat mass ($P \leq 0.05$) was recorded in experiment 6 in experimental groups E4 (natural pollen supplement in the amount of 3500 mg.kg FM⁻¹) – 29.55 g and in E5 (natural pollen supplement in the amount of 4500 mg.kg FM⁻¹) – 28.51 g compared to the control group (35.82 g).

By evaluating the perceptual representation of abdominal fat from the carcass weight of Ross 308 chickens from all experiments, it can be concluded that the lowest (0.57%) was in experiment 10 in group E6 (application of 2% pumpkin pulp. 100 kg FM⁻¹) and the highest (2.25%) in experiment 6, in group E3 (application of natural pollen in the amount of 2500 mg.kg FM⁻¹).

From the point of view of the significant differences ($P \leq 0.05$) in the individual experiments and observed groups, we can conclude that the added feed supplements did not negatively affect the percentage of abdominal fat from the carcass of Ross 308 chickens between the experimental and control groups, except for 10th and 11th experiment, where it was

significantly highest ($P \leq 0.05$) after 3% addition of grape pomace (0.55% – 10th experiment) and after highest used application of grape seeds polyphenols (1.36% – 11th experiment).

Rather, our conclusions refute the conclusions of other authors, who often state that after the application of various natural feed supplements, the proportion of abdominal fat from the chicken carcasses increase. The findings of other authors who have investigated the addition of probiotics, bee products, phytoadditives, various plant by-products and other natural feed supplements are discussed in the following paragraphs.

According to **Al-Khalifa et al. (2019)** the use of probiotics and prebiotics in chicken feed can improve the immune status of the flock by reducing harmful microbes in their intestine and thus reducing the use of antibiotics. However, the authors did not reveal any significant effect on performance of broiler chickens compared with those that did not receive additional probiotics and prebiotics into their diet.

The experiment of **Rehman et al. (2020)** was carried out to evaluate the effects of dietary addition of probiotics (Protexin) and prebiotics (active MOS – mannan oligosaccharides) on growth performance, carcasses, and antibody titer in Ross 308 broilers. In this experiment, authors formulated diets by using 3 levels of probiotics (0, 1, and 2 g.kg⁻¹ of FM) and 3 levels of MOS (0, 1, and 1.5 g.kg⁻¹ of FM) during a 35-day fattening process. Feed intake and feed conversion ratio was improved due to the main effect of probiotic or MOS, but no significant effect was observed for weight gain in the starter, finisher, and overall phases. Apart from carcass yield percentage, no interaction or individual effect of probiotics and prebiotics was observed for carcass, breast, thigh, heart, liver, and gizzard weight. Authors revealed similar carcass weight on average of 946.3 g; as so for abdominal fat weight (29.4 g) and its proportion from the carcass weight 3,11%, what is higher compared with our results of abdominal fat proportion. In spite of the authors's results, it may be concluded that the use of prebiotics and probiotics in broiler diets can improve the growth rate.

Another study was conducted to examine the effects of dietary supplementation with or without *Bacillus subtilis* on carcass traits, meat quality, amino acids, and fatty acids of broiler chickens. Chickens received basal diets without (CN group) or with 500 mg.kg⁻¹ of BS (BS group) for 42 days. The results showed that the breast muscle (%) was higher in BS than in CN ($P \leq 0.05$), while proportion of abdominal fat decreased ($P \leq 0.05$) in BS group (1.52%) compared with CN group (1.74%), what is in contradiction with the results if 1st, 2nd, and 3rd experiments with other types of probiotics, as we revealed increase of abdominal fat proportion in experimental groups. Dietary supplementation with *B. subtilis* could improve the carcass traits

and meat quality of broilers, which is beneficial for the consumers due to the improved fatty acid profile and amino acid composition revealed by (Tang, Liu and Liu, 2021).

On the other hand, in study of Souza et al. (2018) the application of probiotics composed of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium bifidum* and *Enterococcus faecium* did not influence the carcass yield and commercial cuts (breast, drumstick + thigh, and wings). However, the yield of drumstick + thigh was reduced when chickens were raised in environment with higher challenge. No significant effect was also observed with probiotics or environmental conditions on the chemical composition of the carcass. Despite these results, authors conclude that the probiotic does not promote satisfactory improvements, regardless of the environmental challenge used in this study.

In study of Haščík et al. (2019) was evaluated meat performance in chickens Ross 308 after the addition of bee pollen and propolis in a combination with probiotic into feed mixture and drinking water on daily basis, respectively (group E1 supplemented with 400 mg bee pollen extract and 3.3 g probiotic (*L. fermentum*) and group E2 supplemented with 400 mg propolis extract and 3.3 g probiotic (*L. fermentum*). The findings of the work on the meat performance and carcass characteristics of chickens revealed that bee pollen in combination with probiotic was the most suitable feed supplement. Authors found the positive effect after supplementation in group E1 as it was highest (1714 g) compared to control group (1629 g) ($P \leq 0.05$). However, in this group was also recorded the highest amount ($P > 0.05$) of abdominal fat – 25.01 g compared with control group – 22.14 g.

The study of Nemauluma et al. (2023) was conducted to determine the effect of bee pollen inclusion on performance and carcass characteristics in Ross 308 broiler chickens (4 treatments groups – inclusion levels of 0, 4, 8, or 12 g.kg⁻¹ of feed mixture, respectively) in a randomized complete block design with sex as a block having 3 replicates with 10 chickens per replicate. After 21 d, the chickens remained in their treatment groups and fed standard grower diet. The results of this study revealed that bee pollen inclusion had positively improved selected zootechnical parameters in both sexes. Furthermore, carcass weight in both sexes was improved ($P \leq 0.05$) by bee pollen; namely after the highest used supplementation level (12 g) in comparison with the control group (males – 1503.7 g vs. 1386.9 g; females 1497.2 g vs. 1360.6 g) inclusion levels.

The results of study suggest that the broiler chicks can utilize bioactive compounds in BP when supplemented in the starter diets and subsequently improve their growth parameters throughout the growing period as well as carcass yield at slaughter age. These positive

improvements could be due to high quality amino acids, essential oils, vitamins, and minerals of BP. Authors suggest the application of 12 g.kg⁻¹ or more in the starter diets to achieve delightful results on growth performance and carcass yield at slaughter age without causing adverse effects on meat physico-chemical properties and sensory evaluation in both male and female broiler chickens (Nemauluma et al., 2023).

The study (Prakatur et al., 2020) was aimed to determine the influence of dietary supplementation with propolis and bee pollen on carcass characteristics and meat quality of Ross 308 broiler chickens. Among the results to compare with, authors found higher carcass weight in the all experimental groups supplemented with bee pollen or propolis compared to control group with no supplementation, what is in agreement with results of our 5th experiment, as we also recorded lower carcass weight after application of bee pollen and propolis into Ross 308 broiler chickens diet.

Positive effect on carcass weight ($P \leq 0.05$) was recorded after addition of phytobiotics containing cumin, mint, clove and anise in amount of 150 mg.kg⁻¹ of feed mixture into diet of Cobb 500 broiler chickens in study of Glamoclija et al. (2017). Cold carcass weight of chickens fed with supplemental phytobiotics was significantly higher – 1948.7 g, compared to the control group of chickens – 1826.8 g.

In study of Aljumaah et al. (2020), the effects of an antibiotic (avilamicyn) and 3 phytobiotic feed additives (Mix-Oil Mint, Mix-Oil Liquid and Sangrovit Extra, were compared. Authors revealed that the best results in term of the lowest proportion of abdominal fat from carcass weight was found after application of avilamicyn (0.77%) compared to Mix-Oil Mint (1.20%), Mix-Oil Liquid (1.20%) and Sangrovit Extra (1%). This is in the contradiction with our results as we recorded lower proportion of abdominal fat after application of phytoadditives in the 9th experiment, although results were not statistically significant. However, despite overall results of this study, dietary supplementation with phytoadditives could effectively compare with that of antibiotic avilamycin in the maintenance of growth performance and improvement in meat characteristics of broilers challenged with *S. typhimurium*.

The disposal of red grape pomace (GP) in landfills and by incineration has negative impacts on the environment. It is, therefore, imperative that alternative and sustainable ways of managing this waste product are identified. Using GP as a source of nutrients and beneficial bioactive compounds in avian diets is a potential waste-reduction and valorization strategy that promotes sustainable agriculture. However, there is limited information on the valorization of GP for this purpose (Kumanda, Mlambo and Mnisi, 2019a).

In spite of the abovementioned, these authors investigated the effect of dietary inclusion of GP in amount 2.5, 4.5, 5.5 and 7.5% on growth performance, blood parameters, carcass characteristics, and breast meat quality traits of Cobb 500 broilers. They did not find any significant differences on carcass weight, however the markedly highest recorded was in the control group (1299.6 g) what is in contradiction with our results of agricultural by-product as we generally recorded generally higher carcass weight in experimental groups.

A similar study was conducted by **Kumanda, Mlambo and Mnisi (2019b)** after application of pre-treated GP with polyethylene glycol (PEG) and a cellulolytic enzyme mixture on several parameters of Cobb 500 broiler chickens' carcasses. The results showed that the highest achieved carcass weight ($P \leq 0.05$) in this experiment was also in the control group (1276.5 g), what suggest that pre-treatment of polyphenol-rich grape pomace does not positively influence this important carcass parameter.

An experiment (**Aditya et al., 2018**) was conducted to explore the efficacy of grape pomace (*Vitis vinifera*) on growth performance, apparent total tract digestibility of nutrients, blood profile, and meat quality in commercial broilers. The dietary treatments were 1) control, 2) 5 g.kg⁻¹ GP, 3) 7.5 g.kg⁻¹ GP, and 4) 10 g.kg⁻¹ GP supplemented in diets after drying, as was applied GP, flax and pumpkin pomace in our 10th experiment. Highest achieved proportion of abdominal fat (1.72%) was achieved in the fourth group, while the lowest was seen in the control group (1.52%) and first experimental group (1.53%), what is in contradiction with our results of pomace supplementation in 10th experiment. Based on the results of the study, supplementation of GP up to 10 g.kg⁻¹ in the diet of broilers was effective in reducing serum cholesterol and improving meat quality parameters in broilers without affecting growth performance, nutrient digestibility, and carcass traits.

Another study of **Ebrahimzadeh et al. (2018)** investigated the effects of grape pomace and vitamin E on the performance, antioxidant status, immune response, gut morphology and histopathological responses in Ross 308 broiler chickens. In conclusion, the results from this study suggest that supplemental GP up to 10 percent did not significantly influence performance of broiler chickens. Achieved carcass weight ranged from 1492.8 g (10 % GP) to 1618,7 g (control group); abdominal fat weight was the highest after supplementation with 7.4% GP (38.84 g), while its proportion from carcass weight was highest in the control group (2.38%), what is in agreement with our results of 10th experiment as we also recorded the highest proportion of abdominal fat in the control group.

The addition of GP in the broiler diets could increase the immune responses and reduce the feed cost per kg of live weight. The physiological effects of polyphenols depend on many different factors and more research will be needed in the future to establish appropriate dosages of grape polyphenols to optimize health benefits and minimize possible negative effects (**Ebrahimzadeh et al., 2018**).

The study by **Sen and Basalan (2022)** was carried out to investigate the effects of inulin and grape pomace addition to broiler diets on Ross 308 broiler chicken performance, carcass yield and other parameters. Chickens were divided into control group and experimental groups that received supplemented feed mixture with 10 g.kg⁻¹ inulin, 50 g.kg⁻¹ grape pomace, and 10 g.kg⁻¹ inulin + 50 g.kg⁻¹ grape pomace. Authors claim that the significantly highest carcass weight ($P \leq 0.05$) was after combined supplementation (1498.7 g), while the lowest was after grape pomace supplementation (1237.6 g), what is in contradiction with our results of 10th experiment in groups supplemented with pure red grape pomace. As a result, authors claim that grape pomace up to 5% and inulin up to 1% in broiler diets can be used separately as antioxidants. However, grape pomace may adversely affect food conversion ratio depending on the polyphenol level. In addition, it should be considered that they may have a negative effect on immunity when used in combination.

The study of **Zajac et al. (2020)** determined the effect of the addition of 15% of camelina, flax, and sunflower seeds to iso-caloric and iso-nitrogenous diets for broiler chickens during 21 – 42 days of age on the several traits of broiler chickens' carcass and meat quality. Bird carcasses from the full-fat oilseed treatments were characterized by higher breast muscle content and greater gizzard weight as well as a smaller proportion of abdominal fat, that was the highest in the control group (18.1 g) compared to camelina (7.5 g), flax (9.5 g), and sunflower (14.3 g) supplementation, what is in an agreement with our 10th experiment in groups supplemented with flax pomace into broilers diet ($P \leq 0.05$). According to the results of this study, it can be summarized that oily components such as the camelina, flax, and sunflower seeds can be regarded as good dietary components with positive effects on the dietary value of poultry meat.

Among other feed supplements, in study of **Gungor and Erener (2020)**, the effect of raw sour cherry kernel (RC) and fermented sour cherry kernel (FC) by *Aspergillus niger* on growth performance, carcass traits and meat quality in broiler chickens was investigated. The Ross 308 broiler chickens were fed on a basal diet (control) and basal diet supplemented with RC or FC at the 1, 2, and 4% level. Authors did not find any significant differences in the

proportion of abdominal fat (0.4 – 0.5 % from the live body weight), as so for the other carcass characteristics. However, based on the overall results of this study, it can be stated that FC can be used in broiler nutrition up to 2% level although RC can be added to broiler diets up to 1% level without a detrimental effect on growth performance. Dietary inclusion of 1% RC or FC can be recommended due to the positive effects on broiler chickens, namely on growth performance of broiler chickens during fattening process.

The study of **Jalveh et al. (2020)** was aimed to evaluate the effects of dilution of broiler diet with cracked maize on performance and intestinal morphology. Diets of Ross 308 broiler chickens were differing in cracked maize levels. The gradual dilution of broiler chicken diets with cracked maize from 6 (grower diets) up to 25% (in finisher diets) is feasible without any negative effect on productivity, with even a positive effect on feed conversion ratio. Among the carcass traits, inclusion of cracked maize led to lower carcass yield and significant decrease in gizzard proportion. Abdominal fat proportion was not significantly different, ranging from 1.75 to 1.95%, what is similar to results of our studies. The overall positive effect of cracked maize addition into the broiler chicken diet in this study was due to higher particle size, which had a beneficial effect on the entire digestive system morphology.

Another study was conducted by **Infante-Rodríguez et al. (2020)** to evaluate the effect of increasing levels of dietary crude protein (CP) on productive performance, carcass characteristics, and chemical composition of breast and thigh meat in broiler chickens raised in the dry subtropics of northeastern Mexico. Treatment diets for starter and finisher phases had different crude protein concentrations. Broiler chickens fed 21.4% and 18.5% CP in starter and finisher diets, respectively, had better feed conversion ratio than broilers fed the lower CP diet. Carcass characteristics showed minimal influences of dietary CP level and without significant differences. Carcass weight was on average level of 2283.8 g; weight of abdominal fat was lowest in the control group (19.63 g) and on average higher in the experimental groups – 23.49 g, same as for its proportion from carcass weight – 0.83% in control group and 1 – 1.10% in the experimental groups.

Prihambodo et al. (2021) examined the influence of dietary flavonoids on the growth performance, blood and intestinal profiles, and carcass characteristics of Ross broilers. Dietary flavonoids increased the average daily gain of broilers in the finisher phase. There was a reduction in the feed conversion ratio of the broilers both in the starter and finisher phases. The mortality rate tended to decrease with the addition of flavonoids, while the carcass parameters as abdominal fat was generally not influenced, what is in contrast namely with the

results of our 11th experiment aimed at the effect of grape seeds polyphenols as we found significantly highest weight and proportion of abdominal fat after the highest addition of grape seeds polyphenols.

Our results of 9th experiment with various phytoadditives are in agreement with study of **Arczewska-Włosek and Świątkiewicz (2012)**, who investigated the anticoccidial efficacy of supplementing Ross 308 broiler chickens feed mixtures with herbal extract blend containing garlic (*Allium sativum*), sage (*Salvia officinalis*), echinacea (*Echinacea purpurea*), thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) extracts in broiler chickens experimentally infected with sporulated oocysts of *Eimeria acervulina*, *E. tenella*, *E. maxima* and *E. necatrix*. Similar to our results, authors found the highest proportion of abdominal fat ($P \leq 0.05$) in the control group (2.27%) compared to four experimental treatments (1.83, 1.81, 1.84 and 1.51%).

A study was conducted by **Onibi et al. (2009)** to assess the effect of dietary garlic (*Allium sativum*) supplementation on the performance and meat quality of Shaver Starbo chickens. The control diet was the basal diet without garlic supplementation; while experimental diets were supplemented with raw and boiled garlic powder at 500 and 5,000 mg.kg⁻¹ diet, respectively. Supplementary garlic in the diets of broilers did not influence significantly carcass and organ characteristics but the weights of the abdominal fat were numerically lowered. as was also seen in some of our experiments.

Other study (**Ševčíková et al., 2006**) was aimed at the effect of dietary supplementation of selenium in an organic form on performance, carcass traits and selenium content in tissues of broiler cockerels Ross 308. In experimental groups Se-enriched yeast or Se-enriched alga Chlorella was applied as a Se source. Authors claim that no significant differences between the groups were found in carcass traits and dressing percentage. However higher abdominal fat weight was recorded in the experimental groups (Se-enriched yeast – 15.5 g and Se-enriched alga Chlorella – 15 g) compared to the control group – 13.2 g.

5 CONCLUSION

Abdominal fat, despite its favorable composition of fatty acids, is considered one of the wastes in the slaughter processing of broiler chickens. Since 2006, after the ban on feed antibiotics, various natural substitutes have been researched, which also have different effects on the formation of abdominal fat. This scientific monograph was therefore aimed at examining the effect of different feed additives on abdominal fat formation, finding the following:

- In experiment 1, the percentage of abdominal fat content from the carcass in the experimental groups was at most 2.01% compared to the control group (1.73%).
- In experiment 2, it was the highest in the experimental group (1.68%) versus 1.38% (control).
- In experiment 3, it was the highest in the experimental group (1.60%) versus 1.49% (control).
- In experiment 4, it was the highest in the control group (1.65%) compared to the experimental groups (1.52%).
- In experiment 5, it was the highest in the experimental group with propolis addition (1.61%) versus 1.72% (control) and 1.76% (pollen addition).
- In experiment 6, it was the highest in the control group (2.18%) compared to the group with pollen application (1.81%).
- In experiment 7, it was the highest in the group with the application of propolis and probiotics (1.42 – 1.43%), slightly lower in the control group (1.37%) and the lowest with the application of pollen (1.34%).
- In experiment 8, it was the highest with the application of propolis (1.40%), lower with the application of pollen and probiotics (1.32 – 1.33%) and the lowest in the control group (1.28%).
- In experiment 9, after the application of phytoadditives in the nutrition of Ross 308 chickens, it was the highest in the control group (1.12%) compared to the experimental groups (0.94%).
- In experiment 10, after the application of grape, flax and pumpkin pomace, the highest proportion of abdominal fat from the carcass of chickens was after the application of grape pomace (0.84%), followed by the control group (0.74%),

and lower values were recorded when pumpkin and flax pomace were applied (0.59 – 0.65%).

- In experiment 11, it was the highest in the experimental group with the application of grape seed polyphenols (1.36%) compared to the control group (1.20%).

Based on the results of the scientific monograph experiments (n=11), we can state that the application of natural feed supplements based on probiotics, bee products (pollen, propolis, propolis extract, pollen extract), phytoadditives, grape, flax and pumpkin pomace and grape seeds polyphenols did not have a negative effect ($P \geq 0.05$) on the percentage of abdominal fat content from the carcass weight of Ross 308 chickens.

Abstrakt v cudzom jazyku

The aim of the scientific monograph was to investigate the impact of the application of various additions of natural feed additives based on probiotics, bee products, or of their extracts, phytoadditives, grape, flax and pumpkin pomace and polyphenols from grape seeds into complete feed mixtures in the fattening of the hybrid combination of Ross 308 chickens. At the beginning of the experiment, one-day-old chickens of the hybrid combination Ross 308 were placed in boxes, fed and watered according to the *ad libitum* system, while in the experimental groups, unlike the control group (C), various natural supplements were added to the complete feed mixture. In the scientific monograph, the effect of selected supplements on carcass weight, abdominal fat mass and its percentage from the processed carcass of chickens was monitored. The weight of the slaughtered body of Ross 308 chickens was positively affected ($P \leq 0.05$) after the application of *Enterococcus faecium* probiotic (experiment 2), after the application of three probiotics in experiment 3 and in experiment 6 in experimental group E5 with the addition of pollen in the amount of $4500 \text{ mg} \cdot \text{kg}^{-1}$, otherwise, we did not observe significant differences ($P \geq 0.05$) between the experimental groups and the control group in the remaining results of 11 experiments. In experiment 1, the percentage of abdominal fat content from the carcass in the experimental groups was at most 2.01% compared to the control group (1.73%). In experiment 2, it was the highest in the experimental group (1.68%) versus 1.38% (control). In experiment 3, it was the highest in the experimental group (1.60%) versus 1.49% (control). In experiment 4, it was the highest in the control group (1.65%) compared to the experimental groups (1.52%). In experiment 5, it was the highest in the experimental group with propolis addition (1.61%) versus 1.72% (control) and 1.76% (pollen addition). In experiment 6, it was the highest in the control group (2.18%) compared to the group with pollen application (1.81%). In experiment 7, it was the highest in the group with the application of propolis and probiotics (1.42 – 1.43%), slightly lower in the control group (1.37%) and the lowest with the application of pollen (1.34%). In experiment 8, it was the highest with the application of propolis (1.40%), lower with the application of pollen and probiotics (1.32 – 1.33%) and the lowest in the control group (1.28%). In experiment 9, after the application of phytoadditives in the nutrition of Ross 308 chickens, it was the highest in the control group (1.12%) compared to the experimental groups (0.94%). In experiment 10, after the application of grape, flax and pumpkin pomace, the highest proportion of abdominal fat from the carcass of chickens was after the application of grape pomace (0.84%), followed by the control group (0.74%), and lower values were recorded with the application of pumpkin and flax pomace (0.59 – 0.65%). In experiment 11, it was the highest

in the experimental group with the application of grape seed polyphenols (1.36%) compared to the control group (1.20%). Based on the results of 11 experiments of the scientific monograph, we can conclude that the application of natural feed supplements did not have a negative effect ($P \geq 0.05$) on the percentage of abdominal fat content from carcass weight of Ross 308 chickens.

Key words: nutrition, broiler chickens, performance, abdominal fat, natural supplements

Abstrakt v slovenskom jazyku

Cieľom vedeckej monografie bolo skúmanie vplyvu aplikácie rôznych prídavkov prírodných kŕmnych aditív na báze probiotík, včelích produktov, resp. ich extraktov, fytoaditív, hroznových, ľanových a tekvicových výliskov a polyfenolov z hroznových semienok do kompletných kŕmnych zmesí vo výkrme hybridnej kombinácie kurčiat Ross 308. Na začiatku pokusu boli jednodňové kurčatá hybridnej kombinácie Ross 308 umiestnené do boxov, kŕmené a napájané boli systémom *ad libidum*, pričom v pokusných skupinách na rozdiel od kontrolnej (C) im boli do kompletnej kŕmnej zmesi pridané rôzne prírodné doplnky. Vo vedeckej monografii bol sledovaný vplyv vybraných suplementov na hmotnosť jatočného tela, hmotnosť abdominálneho tuku a jeho percentuálny podiel z jatočne opracovaného tela kurčiat. Hmotnosť jatočne opracovaného tela kurčiat Ross 308 bola pozitívne ovplyvnená ($P \leq 0.05$) po aplikácii probiotika *Enterococcus faecium* (experiment 2), po aplikácii troch probiotík v experimente 3 a v experimente 6 v pokusnej skupine E5 s prídavkom peľu v množstve $4500 \text{ mg} \cdot \text{kg}^{-1}$, inak zásadné rozdiely ($P \geq 0.05$) medzi pokusnými skupinami a kontrolnou skupinou sme pri ostatných výsledkoch 11 experimentov nezaznamenali. V experimente 1 sa dosiahol percentuálny podiel obsahu abdominálneho tuku z jatočne opracovaného tela u pokusných skupín najviac 2,01 % oproti kontrolnej skupine (1,73 %). V experimente 2 bol najvyšší v pokusnej skupine (1,68 %) oproti 1,38 % (kontrola). V experimente 3 bol najvyšší v pokusnej skupine (1,60 %) oproti 1,49 % (kontrola). V experimente 4 bol najvyšší v kontrolnej skupine (1,65 %) oproti pokusným skupinám (1,52 %). V experimente 5 bol najvyšší v pokusnej skupine s prídavkom propolisu (1,61 %) oproti 1,72 % (kontrola) a 1,76 % (prídavok peľu). V experimente 6 bol najvyšší v kontrolnej skupine (2,18 %) oproti skupine s aplikáciou peľu (1,81 %). V experimente 7 bol najvyšší v skupine s aplikáciou propolisu a probiotika (1,42 – 1,43 %), mierne nižší v kontrolnej skupine (1,37 %) a najnižší s aplikáciou peľu (1,34 %). V experimente 8 bol najvyšší s aplikáciou propolisu (1,40 %), nižší s aplikáciou peľu a probiotika (1,32 – 1,33 %) a najnižší v kontrolnej skupine (1,28 %). V experimente 9 po aplikácii fytoaditív vo výžive kurčiat Ross 308 bol najvyšší v kontrolnej skupine (1,12 %) oproti pokusným skupinám (0,94 %). V experimente 10 po aplikácii hroznových, ľanových a tekvicových výliskov bol najvyšší podiel abdominálneho tuku z jatočne opracovaného tela kurčiat po aplikácii hroznových výliskov (0,84 %), nasledovala kontrolná skupina (0,74 %) a nižšie hodnoty boli zaznamenané pri aplikácii tekvicových a ľanových výliskov (0,59 – 0,65 %). V experimente 11 bol najvyšší v pokusnej skupine s aplikáciou polyfenolov hroznových semienok (1,36 %) oproti kontrolnej skupine (1,20 %). Na základe výsledkov 11

experimentov vedeckej monografie môžeme konštatovať, že aplikácia prírodných kŕmnych doplnkov nemal negatívny vplyv ($P \geq 0.05$) na percentuálny podiel obsahu abdominálneho tuku z jatočne opracovaného tela kurčiat Ross 308.

Kľúčové slová: výživa, brojlerové kurčatá, úžitkovosť, abdominálny tuk, prírodné doplnky

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