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Review

Fermented Products Enriched with Polyunsaturated Fatty Acids in Broiler Chicken Nutrition and Fat Quality of Produced Meat

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Abstract: Broiler chicken meat is the preferred meat among the human population. Broiler meat contains high-quality protein and a low-fat content, alongside a desirable fatty acid profile. A frequent problem in human nutrition is an insufficient PUFA intake in the diet. One possible strategy to increase the dietary intake of polyunsaturated fatty acids (PUFA) in humans is to produce, and thereby enrich, broiler chicken meat with sufficient amounts of essential PUFA. A method to increase the proportion of essential fatty acids in chicken meat is by changing the fatty acid composition of the feed. Feed production via solid-state fermentation using lower filamentous fungi can be used to prepare valuable feed from cereal by-products enriched with important PUFA and pigments and can thus be included as a suitable feed ingredient in the diet of chickens. From previously published studies, it can be concluded that the application of 3–10% of the prepared fermented products to the diet of broiler chickens increased the proportion of essential fatty acids in the fat of the chicken meat and had a beneficial effect on the growth parameters of chickens.

Keywords: fermentation; filamentous fungi; chicken meat; polyunsaturated fatty acids

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Citation: Makiš, A.; Čertík, M.; Klempová, T.; Semjon, B.; Marcinčáková, D.; Jevinová, P.; Marcinčák, S. Fermented Products Enriched with Polyunsaturated Fatty Acids in Broiler Chicken Nutrition and Fat Quality of Produced Meat. *Appl. Sci.* **2024**, *14*, 4327. https://doi.org/10.3390/app14104327

Academic Editor: Anna Lante

Received: 27 March 2024
Revised: 10 May 2024
Accepted: 15 May 2024
Published: 20 May 2024

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1. Introduction

Chicken meat has many desirable nutritional properties, including a high nutrient content and relatively low caloric value [1]. It is primarily the breast and thigh muscles that represent the most valuable parts of the chicken. Chicken meat has a low fat content and cholesterol level and a relatively high concentration of PUFA, which consumers consider a positive and healthy reason to consume chicken meat [2]. In terms of nutritional value, broiler chicken meat is also very attractive because of its high protein, mineral, and vitamin content [3]. High dietetic value, very good meat flavor, and easy digestibility are other characteristics that consumers appreciate and prefer in broiler chicken meat [4].

The fat quality of broiler chickens is significantly influenced by the composition of the feed and especially by the content of individual fatty acids in the feed [5]. On the other hand, intravital factors, such as the breed (the hybrid), sex, age, rearing method, diseases, use of drugs, fatigue, starvation, transport conditions, and stress, have little influence on the composition and quality of the fat content of broiler chickens [6].

Enrichment of broiler meat with a significant amount of PUFA is a suitable way of increasing the PUFA intake by the population in Europe as poultry meat consumption is high in the EU as well as worldwide [7–9]. The nature of lipid digestion in animals has a significant impact on the transfer of fatty acids from feed to animal products. During periods when animals are not starving and have sufficient fatty acids in the feed, fatty acids

are not synthetized in the body but are incorporated directly from the feed into the fatty tissue of the animals. Feeding fat, therefore, has a direct and generally predictable effect on the fatty acid composition of fat in broiler chicken meat [10], and the proportion of specific unsaturated fatty acids in tissues can be influenced simply by increasing their proportion in the feed.

One of the main aims of using biotechnology in animal nutrition is to produce health-promoting active substances using appropriate microorganisms. Important in the production of these substances are the filamentous fungi of the genera *Umbelopsis* sp., *Mortierella* sp., *Thamnidium* sp., and *Cuninghamella* sp., which can produce significant quantities of biologically active substances on various substrates [11], including by-products of the food industry such as bran, meal, and pomace [12]. Solid state fermentation (SSF) enriches the produced waste with significant amounts of PUFA, hydrolytic enzymes, microbial sterols, beta-glucans, and pigments and can thus be utilized as a suitable feed ingredient in animal diets [13,14]. The fatty acid profile of poultry meat can be significantly influenced by feeding. Feeding a fermented product enriched with important active substances (PUFA, microbial sterols, coenzyme Q, and pigments) will increase their proportion in broiler chicken meat [15].

To the best of our knowledge, only a few experiments have studied the preparation of fermented feed via SSF using lower filamentous fungi and its application in broiler nutrition with the aim of increasing the proportion of PUFA in broiler meat. This review was designed to present a summary of our recent studies of the use of biotechnology for the production of high-PUFA fermented cereal products and their subsequent use in broiler chicken nutrition. The solid state fermentation method and microorganisms suitable for PUFA production of fermented products (FP) are described. The effect of FP on the growth parameters of chickens is also presented. Subsequently, the effect of FP on the quality of the obtained meat regarding the fatty acid composition is also described.

2. Fat Quality of Broiler Chicken Meat

The fat of broiler chickens is localized mainly under the skin and intra-corporeally. The fat content of muscles is significantly lower and is highly dependent on the type of meat [16]. The fat composition of chickens confirms that poultry contains a greater amount of unsaturated fatty acids than saturated fatty acids (oleic acid is the dominant fatty acid in all tissues). The term intracellular fat usually refers to the proportion of fat in the intracellular contents of muscle tissue [17]. It represents a vital organic component of cells; it is highly specific in structure and is firmly embedded in the cell mass. Its quantity is relatively constant and is determined by the proportion of fat components forming structures of intracellular membranes. These structures contain mainly membrane-bound phospholipids and lipoproteins with a significant proportion of unsaturated fatty acids. Kishowar et al. [18] report that the predominant saturated fatty acids in the breast muscle of chickens are palmitic acid (PA; C16:0; 21 to 24%) and stearic acid (SA; C18:0; 15 to 17%). The myristic acid (MA; C14:0) content ranged from 0.40 to 1.02%. Of the monounsaturated fatty acids, oleic acid (OA; C18:1; 26.5–30.5%) was the predominant fatty acid followed by palmitoleic acid (C16:1) and gadoleic acid (C20:1). Regarding polyunsaturated fatty acids (PUFA), Ashayerizadeh et al. [19] observed that linoleic acid (LA; C18:2, n-6) was the predominant one, forming 19.5–22.0%. Arachidonic acid (AA; C20:4, n-6) was found in the range of 1.5 to 5.6%. Cao et al. [20] report that the predominant n-3 PUFA was found to be α-linolenic acid (ALA; C18:3) with a range of 1.26 to 1.81%. The least abundant n-3 PUFA was docosahexaenoic acid (DHA, C22:6, n-3) with a range of 0.007 to 0.022%. Eicosapentaenoic acid (EPA, C20:5, n-3) ranged from 0.76 to 1.35%. The n-6/n-3 PUFA ratios ranged from 6.18 to 6.97. Previous studies demonstrated that the amount and composition of fatty acids in abdominal fat, animal tissues, or muscles in broilers was mainly modified by the dietary fatty acid composition [21–23].

3. Biotechnological Preparation of Cereals Enriched with PUFA via Fungal Solid State Fermentation

3.1. Solid State Fermentation

Biotechnological modification of cereals for the natural preparation of functional cereal-derived by-products containing biologically active PUFA is a challenging field of the food/feed industry. One possibility to prepare cereals enriched with PUFA relies on the ability of suitable microorganisms to utilize these materials and convert them to new fermented products with a high content of valuable metabolites during a process known as solid state fermentation (SSF) [24,25]. SSF is a process in which microorganisms grow on a moist, solid substrate in the absence of free water [26,27]. SSF simulates fermentation reactions occurring in nature and allows microbial utilization of raw agro-materials or by-products of the agro-food industries. As a result of microbial growth and metabolism, various types of value-adding microbial substances accumulate in the fermented food/feed including PUFA, pigments, sterols, organic acids, alcohols, esters, and enzymes [24]. An advantage of the SSF process is that fermented materials can be directly used for food/feed application without any extensive downstream process. Other advantages of SSF are low wastewater production, higher availability of oxygen, a reduced risk of bacterial and yeast contamination, absence of foaming problems, etc. Therefore, such fermentation is also attractive because of its low cost [28].

3.2. Microorganisms for Cereal Utilization

One of the key factors for a successful SSF process is the selection of appropriate microorganisms that can grow on various cereal substrates and, simultaneously, synthesize a range of compounds in large enough quantities. Therefore, suitable microorganisms should meet the following criteria [29]: (a) sufficient coverage of the cereal substrate surface and penetration into the cereal particles for nutrient utilization, (b) production of necessary enzymes (e.g., amylases, proteases, lipases) for the hydrolysis of polymeric compounds in the substrate, (c) adequate growth at reduced water activity, (d) active biosynthetic machinery for the formation of desired compounds, (e) they must not produce any toxins, and (f) the ability to decrease the amount of anti-nutrient compounds in the substrate. To fulfil these criteria, screening of microorganisms has led to the selection of lower filamentous fungi, especially those belonging to Zygomycetes as the best candidates for SSF processes (Table 1). Amongst them, *Thamnidium elegans*, *Cunninghamella echinulata*, *Cunninghamella elegans*, and *Mortierella* (*Umbelopsis*) *isabellina* have been used as producers of gamma-linolenic acid (GLA) and *Mortierella alpina* as a producer of dihomo-gamma linolenic acid (DGLA), AA, and EPA [14].

Moreover, several *Mucor* strains [25,30] and *Umbelospis isabellina* [31,32] have been described to form both GLA and β-carotene. A variety of cereals and cereal products (e.g., rice bran, wheat bran, oat flakes, peeled barley) [33] and legumes [34] have been tested as useful substrates to achieve fermented products (FP) with a high PUFA content. In all cases, the amount of PUFA in the fermented mass was consistently lower when cereals were used as a sole substrate and/or without any pre-treatment. Therefore, microbial transformation of cereal substrates and legumes into a desired fermented mass requires optimization of the cultivation process.

Table 1. Accumulation of γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), arachidonic acid (AA), eicosapentaenoic acid (EPA) in a cereal-based fermented byproduct (BP) prepared via fungal solid state fermentation by selected fungi utilizing various cereal substrates [14].

Strain	Cereal Substrate	PUFA	Yield [g/kg FP]
Thamnidium elegans	spelt flakes/SMG	GLA	7.2
	wheat bran/SMG/sunflower oil	GLA	10.0
	wheat bran/SMG/sunflower oil/plant extract	GLA	20.0
	crushed corn	GLA	10.0
Mortierella isabellina	barley	GLA	18.0
Cunninghamella elegans	barley	GLA	7.0
	barley/SMG/peanut oil	GLA	14.2
Mucor circinelloides	rye bran/SMG/sunflower oil	GLA	24.2
Mortierella alpina	wheat bran/SMG	AA	42.3
	dehulled millet	AA	44.7
	oat bran	AA	87.0
	crushed sesame seeds	DGLA	21.3
	peeled barley/SMG/linseed oil	EPA/AA	23.4/36.3

GLA—γ-linolenic acid, DGLA—dihomo-γ-linolenic acid, AA—arachidonic acid, EPA—eicosapentaenoic acid, FP—fermented product, SMG—spent malt grains, PUFA—polyunsaturated fatty acids.

3.3. Regulation of the SSF Process for the Production of PUFA-Enriched Cereals

The accumulation and amount of microbial PUFA in fermented cereals depend on the substrate, microorganisms, and cultivation conditions. Solid state fermentation is often carried out with the help of an internal solid support or a matrix (e.g., spent malt grains) that is required for improving the efficiency of respiration and aeration, the elimination of heat formed during the fermentation process, and for the reduction of substrate particle agglomeration [33]. Adequate oxygen availability is also necessary for high-activity enzymes that transform carbon to PUFA. The appropriate moistening of a substrate is a significant factor for optimal fungal growth and the evaporative cooling of the fermentation mass. Water serves as a solvent for nutrients and is also necessary for the intracellular transport of mass and for the physical protection against turgor forces. In addition, proper water activity of the cereal substrate prevents the growth of undesired microorganisms and considerably alters both the formation of air in the mycelium and the yield of PUFA [24,33].

Heterogeneity of the cereal substrate and the need for a well-balanced utilization of nutrients from the cereal substrate is a basic problem of fungal SSF. To increase carbon source availability for microorganisms, either partial hydrolysis (chemical, enzymatic) of the cereal substrate or gradual elevation of the carbon/nitrogen ratio using appropriate carbon source supplementation of the cereal substrate could be performed. Nutritional regulation of SSF also includes the supplementation of cereals with various ions (e.g., Ca^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}) and activators or inhibitors (e.g., isolated from plants) that modify enzyme activity involved in the carbon flow to the target PUFA [29]. Depending on the substrate and cultivation conditions, several fermented cereal products enriched with PUFA have been prepared. Under optimal conditions, the final *Thamnidium elegans*-fermented cereals yielded up to 20 g of GLA/kg of FP [35]. *Mortierella isabellina* sufficiently enriched barley with 18 g of GLA/kg of FP [35]. Supplementation of PUFA precursors in the form of extracellular plant oils was also applied for enrichment of cereals with GLA. Cultivation of *Cunninghamella elegans* on a mixture of barley/spent malt grains/peanut oil led to the production of 14.2 g of GLA/kg of product [36]. Growth of *Mucor circinelloides* on a mixture of rye bran/spent malt grains/sunflower oil yielded up to 24.2 g of GLA/kg of FP [1]. The fungal strain *Mortierella alpina* was also described to accumulate 32 g of AA/kg of bioproduct during utilization of a mixture of corn meal and animal fat [32]. Similarly, *Umbelopsis isabellina* growing on a mixture of corn meal and animal fat formed both 6.4 mg of GLA/g of FP and 45 µg of β-carotene/g of FP [37]. In addition, the growth of

Mucor wosnessenskii was optimized for the simultaneous production of GLA and β-carotene. Utilization of a mixture of oat flakes/spent malt grains (3:1) resulted in 10.7 g of GLA and 260 mg of β-carotene/kg of FP [30]. *Umbelopsis isabellina* utilizing corn meal resulted in a fermented product containing 11.5 mg of GLA/g and 51 µg of β-carotene/g [31]. On the other hand, *Mortierella alpina* converted a mixture of wheat bran/spent malt grains (3:1, *w*/*w*) to a FP with 4.2% AA, dehulled millet to a pre-fermented mass containing 4.5% AA, and cracked barley to a final FP consisting of 4.1% AA [29]. *Mortierella alpina* was also used for enrichment of cereals with DGLA via the addition of sesame seeds to peeled barley (17 g DGLA/kg of FP) [29]. The SSF process has been developed to prepare EPA-rich cereals by *Mortierella alpina*, which rapidly consumes a mixture of peeled barley/linseed oil/spent malt grains (0.5:1:3, *w*/*w*) and simultaneously yields up to 23.4 g of EPA and 36.3 g of AA/kg of FP [29]. Thus, such a strategy allows for the preparation of oils with a desirable n-6/n-3 PUFA ratio, finally leading to an increased number of beneficial applications and fermented cereals containing PUFA that have been tested as food additives [25] and as a source of nutrition in ruminant [14,38,39], laying hen [25], and chicken diets [40–42].

4. Fermented Products in Chicken Nutrition

In the last ten years, in part due to the development of green energy production in bioelectric power plants, the price of basic raw materials (wheat, maize) used for chicken feed production has increased. Therefore, cheaper sources are being sought for to fully replace them [43]. One possibility is the utilization of alternative crops [44] or by-products such as bran, spent grains, fruit peels [30,45,46], and oilseed pomace [19] that are produced during food production. These alternatives are more economically viable compared to the main cereals but they contain insufficient protein, high amounts of fibre, and often anti-nutrients, limiting their use in broiler chicken fattening [47]. Several works confirm that the use of fermentation processes either by lower filamentous fungi or bacteria will increase the total protein content and reduce the content of indigestible fibre, antinutrients, and toxic substances in raw materials used for fattening [37,45,47,48]. Thus, the prepared pre-fermented product can be efficiently used for broiler chicken nutrition. The most common problem with non-traditional raw materials in poultry nutrition is the high fiber content, which can be significantly reduced by fermentation; specifically, the indigestible part of fiber can be fermented into digestible fiber [47,49]. Via solid-state fermentation (SSF) without access to free water in the substrate and with the help of the filamentous fungus *Trichoderma pseudokoningii* (a producer of the enzymes xylanase and cellulase), a fermented product was prepared from wheat bran and fed to broiler chickens [45]. Fermented wheat bran contained significantly more enzymes (cellulase and xylanase) and also reduced sugars after fermentation. The addition of fermented bran, replacing corn at 10% in the chickens' diet, did not cause a reduction in the weight gains of the chickens and, on the contrary, significantly reduced feed consumption, which was reflected in a significantly better feed conversion rate of the chickens in the experimental group. *Trichoderma* spp., as the most consistent and safe fungi, are used for the synthesis of cellulase and hemicellulases [50,51]. Fungi have a highly effective enzymatic system that allows them to break down lignocellulosic materials [52]. SSF, using fungi of the genus *Trichoderma* spp., can increase the utilization of a non-traditional raw material (such as bran) in broiler chicken diets; however, they are only able to break down fiber, increase the amount of reducible sugars, and partially increase the total protein content. This genus is not able to produce fatty acids or influence the fatty acid composition of the fermented raw material.

The preparation of feeds based on increasing the proportion of PUFA is based on SSF using lower filamentous fungi [37]. By-products of agricultural origin (spent grain, wheat bran, spelt bran) are used as a substrate and lower filamentous fungi of the genera *Thamnidium*, *Cuninghamella*, *Mortierela*, and *Umbelopsis*, which are able to utilize carbon sources and produce unsaturated fatty acids such as GLA, EPA, and AA, which are used as fermentation agents [14]. The aim of the experiments was to use SSF to prepare complete feed component for broiler chickens that can fully replace a part of the chicken feed mixture

and, in addition, contain a significant amount of fatty acids, which will subsequently increase their proportion in the fat of chicken meat (Tables 2 and 3).

In one of the first experiments, the effect of a fermented product prepared by SSF using the lower filamentous fungus *Thamnidium elegans*, was investigated [53]. Wheat bran was used as an effective substrate for SSF [54]. Since *Thamnidium elegans* is mainly a producer of GLA, the FP was mainly enriched with this specific fatty acid. This fermented feed was added at a dose of 3% to the feed of Cobb 500 hybrid broiler chickens from the 20th day of fattening. The results of the experiment confirmed the positive effect of the addition of the FP on the growth parameters of the chickens. Lower feed consumption, higher final weight, and better feed conversion was observed compared to the control group.

In another experiment, broiler chickens of hybrid Cobb 500 were fed 5% fermented feed [40]. The FP was prepared via SSF fermentation using the lower filamentous fungus *Cunninghamella elegans*; however, spelt bran was used as the substrate. The product produced was energetically superior to commercial feed (CF), containing more fibre but mainly GLA (8.5%). The FP was added to the feed of chickens from day 20 of fattening, with a total fattening period of 35 days. The results clearly showed a significantly lower feed consumption in the experimental group ($p < 0.05$), higher final weight, and better feed conversion rate.

In further conducted experiments, the proportion of fermented feed was increased to 10%, reducing the amount of CF by this amount. The FP were prepared with a higher proportion of GLA and betacarotene or GLA, ALA, EPA, and AA. In one of the experiments, the fermented feed was prepared by fermenting corn meal (waste from corn processing) using the lower filamentous fungus *Umbelopsis isabellina* [55]. The fermented feed was fed to Cobb 500 hybrid broiler chickens from day 10 to day 38 of fattening. Replacing 10% of the CF with the fermented product did not have a negative effect on growth parameters. Feed consumption was slightly lower in the experimental group, but feed consumption and conversion were statistically insignificantly higher. A positive effect of the addition of the FP on the haematological and immunological parameters of chickens was also recorded [56]. Lower total and LDL-cholesterol values were measured in blood samples of the experimental animals ($p < 0.05$). Significantly higher levels of the haematocrit and haemoglobin and a lower number of eosinophils and basophils were recorded in the experimental group ($p < 0.05$). The experimental group also showed higher numbers of B lymphocytes and a greater phagocytic capacity ($p < 0.05$) [41,57].

The effect of adding the FP in a concentration of 10% alone and in combination with agrimony extract (0.2%; *Agrimonia eupatoria*, L.) on growth parameters of chickens was investigated in another experiment [58]. The fermented product was prepared by fermentation of wheat bran using the lower filamentous fungus *Cuninghamella echinulata* [25]. Although replacing 10% of the CF with the fermented product had reduced feed consumption ($p < 0.05$), final chick weights and gains were not affected in the experimental group as reflected in the slightly better feed conversion rate compared to the control group (1.57 and 1.60; $p > 0.05$). A more pronounced effect on gain and final weight of chicks was observed with the feeding of a FP with agrimony extract, which led to the highest recorded final weight of chicks and lower feed consumption as well as feed conversion rate compared to the control group. On the contrary, feed consumption was higher ($p < 0.05$) compared to the experimental group when the FP was fed alone. These experiments also confirm that SSF using lower filamentous fungi can recover by-products and prepare a complete feed component for broiler chicken diets.

A new type of fermented product with a higher proportion of essential fatty acids (GLA, alpha-linolenic acid—ALA, arachidonic acid—AA, and eicosapentaenoic acid—EPA) was prepared in another experiment. The *Mortierella alpina* strain was used for fermentation as a producer of AA and EPA. Wheat bran was used as a substrate for fermentation; to increase the production of EPA in the feed, 1% linseed oil was used. The resulting product contained 2.19% GLA, 6.67% ALA, 0.33% EPA, and 4.93% AA [59].

Table 2. Microorganisms used for SSF for the production of FP and their effects on the growth performance and health parameters of chickens.

Microorganism	SSF Substrates	Results	Reference
Umbelopsis isabellina	corn meal	Influenced the biochemical, hematological, and immunological parameters of chickens	[56]
Thamnidium elegans	wheat bran	Higher final weight, reduced feed consumption, better feed conversion ratio	[53]
Trichoderma pseudokoningii	wheat bran	Reduced feed consumption, which was reflected in a significantly better feed conversion rate of the chickens	[45]
Cunninghamella elegans	spelt bran	Higher final weight, lower average daily feed intake, feed conversion ratio	[40]
Trichoderma viride	copra meal	Growth and feed conversion ratios of chickens were the same as in birds fed the control diet	[60]
Cunninghamella echinulata	wheat bran	Lower average daily feed intake and total feed consumption	[58]
Acremonium charticola and *Rhizopus oryzae*	cassava pulp	Immproved immune response of chickens	[61]
Umbelopsis isabellina	wheat bran	Positive influenceon the gut microbiota and immunity of broilers.	[41]

Table 3. Microorganisms used for SSF for the production of FP and their effects on the fat quality of produced meat.

Microorganism	SSF Substrates	Results	Reference
Umbelopsis isabellina	cornmeal	The amount of GLA, ALA, and OA in the fat of breast muscles was increased and the n-6/n-3 ratio was significantly decreased	[62]
Cunninghamella elegans	spelt bran	Fat of meat contained a higher amount of unsaturated fatty acids, reflected mainly in the amount of ALA and GLA	[40]
Cunninghamella echinulata	wheat bran	Enhanced amount of GLA in the fat of produced meat	[58]
Thamnidium ellegans	wheat bran	Resulted in a significant increase of GLA, DGLA, and AA in the lipids of the breast muscle	[53]
Mortierella alpina	distiller's dried grains with solubles and soybean meal	Increased the PUFA content as well as the proportions of n-6 and n-3 in chicken breasts and the liver.	[63]

The fermented product was added to the experimental group's feed at a rate of 10% and the proportion of commercial feed was reduced by this amount. The FP was fed mainly to increase the proportion of the above-mentioned fatty acids in the fat of the meat produced (the breast and thigh muscles). The results confirmed that the FP lowered feed consumption and that the gains of chickens during fattening as well as feed conversion rate were lower in the experimental group than in the control group ($p > 0.05$). Lower body yield was also recorded. Despite the less favourable results, it can be concluded that this type of FP was also able to replace 10% of the CF without showing significant statistical differences in the growth parameters of the chicks. In addition to the higher proportion of significant fatty acids itself, the fermented product also contained significantly higher concentrations of enzymes, coenzyme Q, and, compared to unfermented wheat bran, a

lower proportion of indigestible fibre and a higher proportion of fat and protein, which is attributable to the fermentation process.

All presented experiments proved that SSF with lower filamentous fungi can produce a complete fermented feed from the by-products with a positive effect on the growth parameters of chickens and serve as a source of important fatty acids.

5. Fermented Products and Quality of the Fat of Broiler Meat

The long-term goal of the experiments is to test multiple strains and substrates in order to develop a fermented PUFA-rich product that, when fed to chickens, will increase the content of important PUFA in the fat of the meat produced (Table 3).

In the first experiment, the fermented product was prepared via SSF using the lower filamentous fungus *Thamnidium ellegans* with wheat bran as the basic substrate [53,64]. The fermented product contained 15% GLA and showed a lower linoleic acid (LA) content compared to the commercial feed (CF). After mixing 3% of the fermented product into the compound feed, there was an increase in GLA content and a decrease in the amount of LA in the resulting feed. There were two experimental groups in the experiment. The first group was fed only the fermented product and the second experimental group was fed, in addition to the 3% fermented product, a 0.1% extract of agrimony (*Agrimonia eupatoria*, L.), which was added to the water. The results of the fatty acid composition confirmed the positive effect of the addition of the FP on the fatty acid composition of the fat of the breast and thigh muscles. The content of GLA, which was supplied in increased amounts in the feed, was significantly increased ($p < 0.05$) in the experimental groups compared to the control group. The increase in the amount of dihomo-gamalinolenic acid (DGLA) in the fat of the meat of the experimental groups was also positive. A significant increase was observed mainly in the breast muscles. However, in the experimental group with the addition of agrimony extract, this increase was lower compared to the experimental group without the addition of agrimony extract. The increase in GLA and DGLA was reflected in the increase of total n-6 PUFA in both breast and thigh muscles of both experimental groups and, consequently, in the evaluation of the total PUFA content, where a significant increase was observed, especially in the breast muscles. We also observed a positive increase in EPA and DPA in the fat of meat of the experimental group. When the n-6/n-3 PUFA ratio was evaluated, this ratio was significantly better in the breast muscles of the experimental groups compared to the control group [53]. The fatty acid composition was slightly different when the fermented product (3%) was fed in combination with agrimony extract (0.1%). In this experimental group, LA, GLA, and ALA formed the highest proportion in the breast muscles compared to the other groups. In the fat of the thigh muscles, DGLA, AA, EPA, DPA, and DHA formed the highest proportion [64]. Particularly valuable is the finding of an increase in just n-3 PUFA after feeding this combination of natural additives. It is the search for appropriate combinations that can ensure an increase in the proportion of important PUFA in the fat of the meat produced. As agrimony also has good antioxidant properties [65], it has been added to the water for chickens in order to improve the oxidative stability of the meat during storage. However, in addition to oxidative stability, plant extracts also affect the fat composition of meat [66].

In another experiment, the FP was prepared via SSF using the lower filamentous fungus *Thamnidium ellegans* with spelt bran as the basic substrate [40]. The product formed via fermentation contained 8.45% GLA and showed lower LA and ALA and higher oleic acid contents compared to the commercial feed mixture. The base ingredient used (cereals) as well as the fermentation conditions played a role in the resultant lower proportion of GLA in the produced substrate and the partial change in the fatty acid profile of the FP [67] compared to the first experiment. The fermented product was added to the chickens of the experimental group in the amount of 5% and the proportion of CF was reduced by this amount. Feeding this FP had a positive effect on the increase of GLA in both breast and thigh muscle fat in chickens of the experimental groups. However, the higher proportion of GLA in the feed did not significantly affect the content of other important fatty acids

(DGLA, AA) in the breast muscle meat and only slightly higher values were recorded in the thigh muscle meat compared to the control group. It is assumed that the ingested GLA from the feed is directly incorporated into the adipose tissue of the chickens and is not utilized in the formation of other fatty acids (DGLA, AA). A statistically insignificant decrease of DPA, EPA, and DHA in the fat of the breast muscles of the experimental group was observed. On the contrary, in the thigh muscles, the proportion of these acids was slightly higher ($p > 0.05$). After feeding the FP to the experimental group, there was an increase in the MUFA and PUFA content and, on the contrary, a decrease in SFA in both the meat and body fat of the chickens. The n-6/n-3 PUFA ratio decreased only in the thigh fat; it remained unchanged in the breast muscle fat.

In another experiment, the effect of a fermented product at a dose of 5% on the fat quality of broiler chicken meat was investigated [68]. The fermented product was prepared via SSF using the lower filamentous fungus *Cuninghamella echinulata* and wheat bran was used as a substrate. The experiment also included verification of the effect of feeding the fermented product in a combination with agrimony extract. Agrimony has strong antioxidant effects and also has a beneficial effect on the gastrointestinal tract, which was hypothesized to result in higher oxidative stability of the meat and better growth parameters of the chickens. The fermented product showed a 7.11% GLA content and a higher proportion of ALA and LA compared to the commercial compound feed and a lower proportion of palmitic acid and myristic acid. The prepared fermented product was fed to the chickens from day 17 of fattening at a dose of 5% (the fattening period lasted 38 days). Feeding of the fermented product had a different effect on the fatty acid profile of breast and thigh muscle fat. The most significant was the increase in GLA in both breast and thigh muscle fat. Skimming the FP caused an increase in oleic acid, LA, and DGLA in the fat of the pectoral muscles. Conversely, there was a decrease in the content of the following n-3 PUFA, EPA, DPA, and DHA, compared to the control group. In the thigh fat, a significant decrease in oleic acid and an increase in DGLA, AA, but mainly n-3 PUFA (EPA, DPA, and DHA) compared to the control group was observed. Coinciding with the first experiment, ingestion of the fermented product in combination with burdock extract administered in water produced a more pronounced effect on the fatty acid profile. In this experimental group, an overall increase in the saturated fatty acid content (myristic, palmitic) and a decrease in the unsaturated fatty acid content was observed in comparison to the experimental group without rapeseed.

In order to obtain meat that may be labelled as a functional food, a higher proportion of essential fatty acids must be achieved. Therefore, it was necessary to prepare a new type of feed and also to increase the ration fed to broiler chickens. To increase the proportion of mainly GLA in the fat of the meat and also to improve the growth parameters of the chickens, a new type of FP containing mainly GLA and beta-carotene was produced. The product was prepared via SSF using the lower filamentous fungus *Umbelopsis isabellina* [56]. *Umbelopsis isabellina* produce oils rich in PUFA, especially GLA [69]. Corn meal was used as a basic substrate, which is a very good source of carbon necessary for fatty acid production. The obtained product contained 3.03 mg g^{-1} of GLA and 3.12 μg g^{-1} of beta-carotene. However, the proportion of GLA in the fat of the feed was low, only 3.5%. In the final product there was a higher proportion of oleic and linoleic acid, which is related to the composition of the FA of the substrate (maize) in which these two fatty acids predominate. The FP was added to the experimental group at a dose of 10%, with the proportion of CF being reduced by that amount. The FP was fed to the chickens from day 11 until the end of fattening (day 39). The results of the fatty acid composition profile of both breast and thigh muscle fat confirmed the tendency to increase GLA in meat fat [62]. However, the effect was not as much as was expected. This was related both to the low proportion of GLA in the feed of the experimental group and also to the fatty acid profile of the commercial feed as well as the fermented product itself. Maize meal was not a suitable substrate to increase the proportion of significant fatty acids via fermentation. However, the fermentation process significantly improved the nutritional parameters of corn meal; therefore, fermentation of

corn by-products to improve their nutritional properties is a good way to recover them and use them for animal nutrition.

Furthermore, due to the failure to significantly increase GLA in meat by feeding a fermented product prepared by the lower filamentous fungus *Umbelopsis isabellina* on cornmeal, a new fermented *Umbelopsis isabellina* product was produced with wheat bran as the substrate. The final product, at a dose of 10%, was fed to chickens separately and in combination with agrimony extract (0.2% *Agrimonia eupatoria*, L.) [58]. The results confirmed the effect of the FP prepared in this way in increasing the proportion of GLA in the fat of chicken meat. The fermented product, at a dose of 10%, also had an effect on the composition of other fatty acids in the fat of the produced meat. The proportion of LA and DGLA increased in the fat of the breast muscles. In contrast, the proportion of stearic acid and PUFA (AA, EPA, DPA, and DHA) decreased significantly. This was also reflected by a slight increase in the proportion of saturated fatty acids and a decrease in n-3 PUFA. Subsequently, the n-6/n-3 PUFA ratio also increased slightly in the experimental group.

All the previous experiments confirmed the possibility of influencing the fatty acid profile via the addition of a FP to broiler chicken feed. Thus, a significant influence on and increase of the proportion of GLA in the fat of the produced meat was obtained. Other PUFA (ALA, DGLA, EPA, DPA, DHA) can also be influenced in the fat of meat by fermented feed, but only if the fermented feed was fed at a proportion of 3 or 5%. At proportions of 10% and higher of the FP, the proportion of important PUFA in the fat of the produced meat began to decline. Therefore, it was necessary to search for a new species of lower fibrous fungus that would ferment cereal by-products and also produce other types of polyunsaturated fatty acids.

Based on the previous experiments of the authors Čertík et al. [29], Semjon et al. [59], and Marcinčák et al. [62], a possibility of the use of an innovative FP in the diet of broilers was examined. The lower filamentous fungus, *Mortierella alpina*, was selected as a substrate for SSF. *Mortierella* is a very good producer of EPA and AA [70]. To improve the production of EPA in the fermented product, linseed oil (a source of alpha-linolenic acid and ALA) was added to the substrate (wheat bran). The prepared FP contained 2.5% GLA, 6.6% ALA, 4.9% AA, but only 0.3% EPA. A significantly higher proportion of PUFA, n-3 PUFA, and a better n-6/n-3 PUFA ratio was also observed. Although *Mortierella alpina* is described as a good producer of EPA via solid state fermentation [29,71–73], which was partly confirmed by test results, good EPA production was not confirmed via fermentation of the product alone. Even after the addition of linseed oil, there was only partial production of this important acid, and the fermented product itself contained only about 0.3% EPA reflected by the fact that there were only traces of EPA in the feed of the experimental group after mixing 10% of the FP into the commercial mixture. Therefore, more testing and improvement of fermentation conditions is needed to significantly increase the EPA production in the final product. Furthermore, due to the more complex fermentation conditions, the use of biotechnology for the production of significant PUFA for large-scale feed production is still limited, as even a small change in fermentation factors (temperature, amount of water, pH, substrate, and nutrient composition) can significantly impact the FA composition of the final product.

Feeding 10% of the above FP in broiler chicken feed only partially affected the fatty acid composition of the chicken meat. A statistically significant increase in the proportion of ALA and GLA in the fat of both breast and thigh muscles can be considered as a significant finding. The increase in DPA content in both breast ($p < 0.05$) and thigh ($p > 0.05$) muscle fat was also positively evaluated. On the contrary, compared to the control group, an increase in the proportion of AA was not observed despite this fatty acid being present in higher amounts in the fermented product as well as in the feed of the experimental group. In addition, despite efforts to increase the proportion of EPA in the fat of the produced meat, there was no change in the proportion of this acid in the fat of the chicken meat and its content was comparable to that of the control group. The proportion of DHA was comparable to that of the control group. The proportion of n-6/n-3 polyunsaturated fatty

acids was reduced only in the breast muscles; in the thigh muscles, it was comparable to that of the control group.

Although there was no desired effect in the FA composition of the chicken fat meat, this type of FP also confirmed that SSF with a lower-filamentous fungus can produce a product rich in important FA from cereal products. By feeding a certain proportion of the product prepared this way, it is possible to partially modify the fatty acid profile of the meat produced and partially increase the proportion of important PUFA in the fat of chicken meat.

6. Conclusions

Fermented products represent a new source of important fatty acids (e.g., GLA, EPA, ALA, AA) useful in broiler chicken nutrition. Various microorganisms have been discovered and described to have an immense production potential. Another positive aspect is the finding that fermented products added to broiler chicken feed at doses of 3–10% either had a positive effect or did not negatively affect the growth parameters of broiler chickens.

Biotechnology represents a potential way of improving cereal by-products for use in chicken nutrition by increasing the content of important PUFA or by producing active substances that improve feed utilization, improve animal health, and, most importantly, increase the content of important PUFA in the fat of the meat produced. SSF is a prospective bioprocess combining both fungal utilization (*Thamnidium elegans*, *Mortierella isabellina*, *Umbelopsis isabellina*, or *Cunninghamella* sp.) of moist solid materials (agriculture by-products) and the production of valuable metabolites via a low-cost approach. The attractiveness of SSF lies mainly in the use of readily available and inexpensive substrates such as cornmeal, spelt, and wheat bran. Thus, SSF produces feed enriched with important PUFA and several active substances that have been shown to have a clear positive effect on the health of broiler chickens.

For the better utilization of agricultural by-products in poultry nutrition using SSF, a combination of several species of lower filamentous fungi in the fermentation process must be studied. It is one of the possible ways of enriching the fermented product with specific PUFAs and enzymes. This will contribute to the use of such FP instead of alternative PUFA sources and serve as a promising way for poultry nutrition to meet the globally increasing demand for essential PUFAs in broiler meat in a sustainable way.

Author Contributions: Conceptualization, S.M. and M.Č.; writing—original draft preparation, S.M., D.M., M.Č. and A.M.; writing—review and editing, T.K. and B.S.; formal analysis, P.J. and D.M. supervision, S.M. and A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Slovak Research and Development Agency under contract no. APVV-18-0039.

Acknowledgments: Thanks to Serena Faith Kishore Amrit, a native speaker from USA, who is a student at the University of Veterinary Medicine and Pharmacy in Košice (Košice, Slovakia), for English proofreading.

Conflicts of Interest: The authors declare no conflicts of interest.

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