




Review

Essential Oils in Broiler Chicken Production, Immunity and Meat Quality: Review of *Thymus vulgaris*, *Origanum vulgare*, and *Rosmarinus officinalis*

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Abstract: The use of essential oils in animal nutrition has attracted attention as a potential substitute for antibiotic growth promoters in the past twenty-five years. This paper will review the current scientific evidence on the usage of essential oils from Lamiaceae family members such as *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano), and *Rosmarinus officinalis* (rosemary) in broiler nutrition in terms of production results, immunity, and meat quality properties. Essential oils are effective in broiler nutrition when incorporated into the diet on a variety of levels, such as dietary composition, level of feed inclusion, and bird genetics. Moreover, the efficacy of essential oils is influenced by many factors, such as the composition of the oil. Due to big differences in the composition and sources of essential oils, comparing different studies using them can be challenging. Therefore, biological effects may differ significantly. Despite this, a great deal of research supports essential oils' potential use as natural, antibiotic-free growth promoters for broilers. Growth promotion mechanisms are still not clearly understood as there is limited information on essential oils' effect on nutrient digestibility, gut function, and the immune system. There is no question that essential oil consumption can reduce pathogen growth in the gut, but their effects on the intricate gut ecosystem as yet remain unclear. This review concludes with further recommendations regarding the application of dietary essential oils in broiler nutrition.

Keywords: essential oils; poultry; chickens; animal nutrition; meat quality; medicinal plants; bioactive compounds



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1. Introduction

Today, the production of broiler chickens is the most intensive branch of animal husbandry since broiler chickens are characterized by very fast reproduction, short breeding periods, and relatively low investment, which, compared to other branches of livestock production [1–3], allow faster turnover and, thus, efficient and economical production. On the other hand, chicken meat is characterized by favorable nutritional composition [4], easy digestibility [5], and high energy value [6], and from an economic aspect, it is food available to all segments of society as it is accepted by all cultures and religions [7–10]. Therefore, the modern production of broiler chickens, as an important part of the chain of food production for the global population, which is increasing every day, aims to meet high legal regulations for quality and safe products for human consumption.

The quality and safety of feed for broiler chickens are not only key factors to meeting production priorities but also increasing production and improving the quality of food of animal origin, such as meat [11,12]. To maximize the genetic potential of chickens, it is

necessary to satisfy their physiological needs, so we should strive for nutritional mixtures to be nutritionally balanced [13]. However, in addition to the basic nutrients, today, an increasing number of dietary supplements are used, which aim to improve benefits to and inhibit harmful effects on the body of chickens [14,15]. For this reason, in recent years, in modern broiler production, more and more attention has been paid to medicinal plants as an alternative to banned antibiotics [16].

Phytobiotics represent a new generation of natural supplements, which include medicinal plants and spices, plant extracts, and essential oils, characterized by numerous biological properties [17,18]. The medicinal and useful properties of essential oils in the diet of broiler chickens are reflected in improved production characteristics [19] as well as the ability to increase the body's immune response [20], which has a positive effect on maximizing the genetic potential of chickens and reducing mortality and, thus, increasing profitability. Additionally, phytobiotics and essential oils exhibit hypocholesterolemic effects by inhibiting the most important enzymes involved in the synthesis of cholesterol and lipids, which significantly reduces cholesterol in the blood and edible tissues and the proportion of abdominal fat in broiler chickens [21]. This fact certainly supports phytobiotics and makes them a significant addition to the diet and production of foods necessary for the nutrition of special categories of consumers. In addition, essential oils increase the digestibility and utilization of nutrients, as well as improve the quality of carcasses and meat of broiler chickens.

The quality of carcasses and meat is a key element of competitiveness in the market as it influences the decision to purchase products [22]. At the same time, quality influences the creation of a positive image of the product by consumers whose expectations are significantly met, as consumers are the last link in the production chain. The carcass quality of broiler chickens is a very complex concept and can be assessed from several aspects [23]. Thus, from the point of view of industry and consumers, slaughtered broiler chickens must have good yields, desirable conformation, as much meat as possible on the carcass, optimal fat distribution, appropriate skin color, and as little carcass damage as possible during production [24]. The presence of certain tissues in the more important parts of the carcass is, without a doubt, the element that determines the quality of the carcass of broiler chickens. In scientific and professional circles, it is accepted that the quality of meat is defined as the sum of all objectively measured nutritional, technological, sensory, and hygienic–toxicological properties, i.e., quality factors [25]. The quality of chicken meat, therefore, implies a large number of properties that determine the suitability of meat for consumption, processing, or storage and depends on several interrelated factors that include the conditions of meat production.

Modern aspects in the production of chicken meat represent a perspective to all countries [26], bearing in mind that the products of animal origin obtained in this way represent a ticket into the world market. In addition, the advantages of such broiler chickens can be seen through the economic aspect since the use of natural feed supplements such as essential oils insignificantly increases the price of feed (because they are added in very small quantities) and, as a result of their use, allows sustainable production of safe foods of animal origin without antibiotic residues and without affecting broiler chicken breeding technology.

Given the importance and relevance of essential oils, it is scientifically justified and interesting to examine the possibilities and effects of using selected natural growth stimulants in the intensive breeding of broiler chickens on production characteristics, the quality of chicken carcasses and meat, the digestibility of nutrients, the fatty acid composition of the meat, and the economic viability of production.

2. Importance of Poultry Meat in Human Nutrition

In the past, meat was considered an essential nutrient for human growth and development. It has been found that excessive consumption of red meat may increase one's risk of developing several forms of cancer and cardiovascular and metabolic diseases; however,

meat consumption has been an important factor in the evolution of the human species, especially in the development of the brain. In European legislation, the term meat refers to the edible parts removed from the carcass of domestic ungulates, including bovine, porcine, ovine, and caprine animals, as well as domestic solipeds, poultry, lagomorphs, wild game, farmed game, and small and large wild game.

Since ancient times, meat has been one of the most important foods in human nutrition [27]. Even today, it is no different, and the demand for meat, especially poultry, is constantly growing [28]. Poultry, which includes chicken meat, is one of the foods of animal origin that are most accessible to the widest layers of consumers. Due to its high protein and low-fat content [29], nutritionists recommend, besides poultry meat, rabbit meat [30] as the healthiest source of protein of animal origin.

Chicken meat is, biologically, a very valuable food with high protein content and favorable amino acid composition as it is characterized by low-fat content with significantly high proportions of polyunsaturated fatty acids (PUFAs), which have become increasingly important in recent years [31,32]. Additionally, the favorable ratio of saturated (SFAs) and unsaturated (UFAs) fatty acids, as well as the low content of total cholesterol (TC), makes chicken meat indispensable in the diet of children, convalescents, and chronic patients, as well as those suffering from cardiovascular diseases [33].

Petracci et al. [34] stated that neutral taste, good texture, and light color are characteristics of this type of meat, which makes it more suitable for processing compared to other types of meat. The listed properties enable producers to optimize the taste and texture of the meat according to the needs of the market and the target groups of consumers.

An important advantage of chicken meat compared to other types of meat is certainly the fact that it is accepted by all cultures and religions [7], while the relatively low price of chicken meat [35] compared to beef, lamb, and pork provides the opportunity to use this highly valuable food in everyday diets [36,37].

3. Essential Oils and Their Bioactive Constitutes

In recent years, Due to the very important biological properties they possess, but also due to their very wide distribution and diversity, essential oils represent an important gift of nature that has intrigued many scientific teams around the world.

It is estimated that there are about 3000 types of essential oils on the market today [38], for the production of which at least 2000 plant species are used, of which 300 essential oils are of commercial importance [39]. Dima and Dima [40] reported that between 40,000 and 60,000 tons of essential oils worth about USD 700 million are produced annually, indicating a significant increase in demand for essential oils worldwide. Essential oils are also recognized as safe for use in the food industry by the Flavor and Extracts Manufacturers Association (FEMA) and the Food and Drug Administration (FDA) and are on the Generally Recognized as Safe lists (GRAS), as reported by Hallagan and Hall [41].

Essential oils are aromatic and easily volatile liquids, mostly colorless or yellowish in color. The consistency of most essential oils is similar to water or alcohol, but some can be sticky and viscous. No essential oil contains fats or oils, although they carry the term “oil” in their name, which is often a misconception about the composition of these heterogeneous mixtures [42]. It is a characteristic of essential oils that they do not dissolve in water or dissolve very little, while they dissolve well in all organic solvents (ether, chloroform, gasoline, etc.). Additionally, essential oils thicken, darken, and react acidically when exposed to air for a long time. It can be said that it is difficult to determine the boiling point of these complex mixtures, given the large number of compounds of which they are composed; the boiling temperature usually ranges between 150 and 280 °C, so individual components can be separated by fractional distillation [43].

Data on methods of isolating essential oils from plant materials and the goals of these first procedures are poorly reported and uncertain [43].

Essential oils from plant material are obtained by applying various methods: hydrodistillation [44], extraction with organic solvents [45], cold pressing [46], extraction with fluids

in the supercritical state [47], extraction with non-volatile solvents [48], and extraction with solvents supported by microwave action [49]. The method that will be used depends on the nature and type of raw material, as well as on the economic viability of the method and the application of the obtained oil.

The yield of essential oils from individual plants or parts of plants usually ranges between 0.2% and 2.0%, with exceptions; the yield of rose oil does not exceed 0.03%, while the yield of clove essential oil reaches up to 20% [50].

Today, essential oils are most often used in the cosmetics [51] and pesticides industries [52], the food [53] and pharmaceutical industries [54], and animal nutrition [19,55–58], given the many positive properties they exhibit in an animal body.

Essential oils are highly concentrated mixtures of a large number of diverse, fragrant, and easily volatile compounds that are localized in different parts of plants and play a key role in the treatment of various diseases in both humans and animals [57]. These are most often flavonoids, polyphenols, tannins, alkaloids, terpenoids, polypeptides, and many other compounds that make individual oils specific [59]. These plant compounds are known as secondary metabolites as they are products of secondary plant metabolism. It is estimated that about 500,000 secondary molecules (SM—small organic molecules produced by an organism that are not essential for their growth, development, and reproduction), which are biologically active compounds, have been identified to date. Although they do not participate in the basic life functions of plants, and although their lack cannot negatively affect the growth and development of plants, biologically active compounds still play important roles in the life cycle of each plant. They are known to be inactivated forms and depots of harmful products and are components of certain enzyme systems (coenzymes); they are also characterized by hormonal activity, protect the plant by preventing infections by bacteria, fungi, and viruses, and protect against an overdose of ultraviolet radiation, excessive transpiration, and other environmental factors. They also play an important role in regulating the plant's communication with the surrounding environment. In recent years, these compounds of plant origin have played an important role in pharmacy, medicine, and animal nutrition, given the antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and many other properties that distinguish them [60].

It is interesting to note that between 20 and 60 different biologically active compounds, present in very different concentrations, may be present in the essential oil of certain plant species [61], with only a few compounds being dominant (20–70%), while others are present in traces. However, the special combination of all these components gives a specific impression, and it is assumed that even the biologically active components present in the traces can be of great importance in the biological activity of certain essential oils. The content of biologically active compounds can vary widely depending on the parts of the plant used (seed, leaf, root, flower, bud, and stem), the season of collection, and the geographical area in which they are grown [62]. Additionally, the procedure used to obtain essential oils can affect the content of biologically active compounds in the final product and, thus, their effectiveness.

3.1. Chemical Composition of Essential Oils Derived from the Lamiaceae Family

Table 1 shows the qualitative and quantitative chemical composition of essential oils of selected plant species *Thymus vulgaris*, *Origanum vulgare* [63], and *Rosmarinus officinalis* [64].

In the analyzed *Thymus vulgaris* oil, the dominant group of compounds was represented by monoterpenes (95.02%), with significantly more oxidized compounds (66.35%) than hydrocarbons (28.67%). Sesquiterpenes (2.54%) were also present in a small percentage of the oil. The analysis of *Origanum vulgare* essential oil identified a total of 43 compounds, representing 98.08% of the essential oil. Comparing the basic groups of identified compounds in the essential oil of *Origanum vulgare*, it was found that the share of monoterpenes (94.67%) was far higher than the share of sesquiterpene compounds (2.52%), while the group of oxidized monoterpenes had high quantitative values of phenolic compounds (66.18%), primarily carvacrol (58.84%).

Table 1. Chemical composition of *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano), and *Rosmarinus officinalis* (rosemary) essential oils.

No.	Components (%)	Essential Oil		
		<i>Thymus vulgaris</i> [63]	<i>Origanum vulgare</i> [63]	<i>Rosmarinus officinalis</i> [64]
1.	tricyclene	0.13	0.06	0.15
2.	α -thujen	0.19	0.25	-
3.	α -pinene	0.76	1.35	12.50
4.	camphene	0.27	0.39	2.85
5.	β -pinene	0.03	0.55	0.36
6.	1-octen-3-ol	0.16	0.14	-
7.	myrcene	0.79	0.87	0.90
8.	α -phellandrene	0.92	0.13	-
9.	Δ -3-carene	0.13	0.10	0.96
10.	α -terpinene	1.25	1.05	-
11.	p-cymene	18.71	19.90	1.80
12.	β -phellandrene	0.48	0.51	-
13.	1,8-cineole	0.61	0.16	16.10
14.	(Z)- β -ocimene	-	0.04	-
15.	(E)- β -ocimene	-	0.02	-
16.	trans-decahydronaphthalene	0.04	-	-
17.	γ -terpinene	4.34	3.11	-
18.	cis-sabinene hydrate	0.14	-	-
19.	terpinolene cineole	0.06	-	-
20.	p-mentha-2,4-diene	-	0.02	-
21.	cis-decahydronaphthalene	0.21	0.03	-
22.	undecane	0.05	0.18	-
23.	linalool	2.52	0.26	4.05
24.	camphor	0.08	0.10	17.70
25.	menthone	0.17	-	-
26.	borneol	0.74	0.58	9.23
27.	terpinen-4-ol	1.27	0.78	2.05
28.	α -terpineol	0.24	-	2.67
29.	trans- dihydrocarvone	0.17	0.12	-
30.	dodecane	0.12	0.39	-
31.	dihydro citronellol	0.11	-	-
32.	methyl ester thymol	0.20	-	-
33.	methyl ester carvacrol	0.49	0.54	-
34.	carvone	0.05	-	-
35.	citrollene	0.03	0.04	-
36.	carvenone	0.18	-	-
37.	bornyl acetate	0.10	-	-
38.	isobornyl acetate	-	0.14	-
39.	tridecane	0.07	0.10	-
40.	thymol	43.20	4.76	-
41.	carvacrol	16.57	58.84	-
42.	thymol acetate	0.03	-	-
43.	carvacrol acetate	0.06	-	-
44.	α -ylangene	0.04	-	-
45.	α -copaene	0.05	-	-
46.	α -cubebene	-	0.08	-
47.	β -bourbonene	0.04	0.05	-
48.	tetradecane	0.05	0.05	-
49.	trans- β -caryophyllene oxide	1.01	1.04	0.21
50.	aromadendrene	-	0.05	-
51.	α -humulene	0.08	0.14	-
52.	γ -muurolene	0.06	-	-

Table 1. Cont.

No.	Components (%)	Essential Oil		
		<i>Thymus vulgaris</i> [63]	<i>Origanum vulgare</i> [63]	<i>Rosmarinus officinalis</i> [64]
53.	germacrene-D	-	0.04	-
54.	γ -amorphene	0.06	-	-
55.	viridiflorene	-	0.05	-
56.	α -muurolene	0.05	-	-
57.	β -bisabolene	0.21	0.63	-
58.	γ -cadinene	0.12	0.04	-
59.	δ -cadinene	0.16	0.10	-
60.	cariofilene oxide	0.53	0.30	-
61.	1,10-di epi cubenol	0.02	-	-
62.	10-epi- γ -eudesmol	0.02	-	-
63.	τ -cadinol	0.05	-	-
64.	cadalene	0.04	-	-
65.	verbenene	-	-	0.99
66.	limonene	-	-	2.96
67.	α -terpinolene	-	-	0.47
68.	phenol	-	-	0.99
69.	verbenone	-	-	13.80

In the analyzed *Rosmarinus officinalis* oil, 19 compounds, which made up 95.60% of the essential oil, were identified. The dominant group of chemical compounds was represented by bicyclic monoterpenes (87.20%), while monocyclic and acyclic monoterpenes were present in concentrations of 3.04 and 3.05%, respectively. The share of sesquiterpene compounds was significantly lower (2.32%) [64].

As can be seen from the chemical composition of the selected plant species of thyme, oregano, and rosemary, terpenoids (or so-called isoprenoids) are one of the most common groups of secondary molecules, i.e., biologically active compounds in these plants [63,64]. Degenhardt et al. [65] reported that this group of biomolecules includes about 30,000 compounds.

Monoterpenes are the most common subgroup of terpenoids present in essential oils isolated from plant material, where they make up to 90.00% of most essential oils and are characterized by a very pleasant mild odor. In their structure, monoterpenes contain two isoprene units, i.e., 10 carbon atoms. This group of compounds includes thymol, carvacrol, α -pinene, γ -terpinene, and 1,8-cineole [66].

Thymol (2-isopropyl-5-methylphenol) is a phenolic derivative of cymene and an isomer of carvacrol. Thymol is characterized by a colorless crystalline structure with a very pleasant odor; it does not dissolve in water but dissolves well in alcohol and other organic solvents. Carvacrol (5-isopropyl-2-methylphenol) is a colorless to pale yellow viscous liquid that does not dissolve in water, dissolving very well in ethanol, ethyl ether, propylene glycol, and bases. Thymol and carvacrol are compounds that inhibit lipid peroxidation, have a digestive-stimulating effect, and are characterized by antioxidant, antispasmodic, diuretic, antiviral, antibacterial, antifungal, and immunomodulatory properties. The presence and high percentage of monoterpene hydrocarbons, p-cymene and γ -terpinene, cannot be observed independently of the presence of thymol and carvacrol since these compounds are their precursors; hence, they mostly occur simultaneously in essential oils [66].

Based on a review of previous research, it has been established that thymol and carvacrol are the most abundant biologically active compounds in thyme essential oil [67]. In these studies, the content of thymol ranged from 43.20% to 59.95% and the content of carvacrol from 2.40% to 16.57%. β -linalool and 1,8-cineole are also present in high concentrations in thyme essential oil, while other components are present in traces [67–69].

Numerous studies indicate that the chemical composition of oregano essential oil does not differ much compared to the chemical composition of thyme essential oil in terms of the most common compounds, with oregano essential oil having a significantly higher carvacrol content and lower thymol content compared to thyme essential oil [70–74].

Based on previous research, it has been established that monoterpene compounds such as camphor, 1,8-cineole, and α -pinene are the most dominant components present in rosemary essential oil. In the research of Šarić et al. [64], the mentioned three compounds accounted for about 46.00% of rosemary essential oil, while their value amounted to as much as 77.50% in the research conducted by Takayama et al. [75]. These biologically active compounds are responsible for antioxidant, antibacterial, antifungal, anti-inflammatory, anticancer, and many other properties attributed to rosemary [75].

Compound 1,8-cineole is a monocyclic ether and monoterpene that is characterized by a fresh, cooling odor [76]. It does not dissolve in water but is miscible with ether, ethanol, and chloroform. α -pinene is an organic compound from the group of bicyclic monoterpenes, and it is also an alkene. It dissolves very poorly in water but is miscible with acetic acid, ethanol, and acetone [76].

The differences in the composition of essential oils are supported by the already mentioned fact that the chemical composition of essential oils in medicinal plants is influenced by many factors. Thus, the geographical origin, climate, and time of collection of plant material, as well as the process of obtaining essential oil from the plant material, affect the type and presence of certain biologically active compounds [43]. Mecherghi et al. [77] investigated the influence of oregano harvest year on the composition of essential oil obtained from this plant material, concluding that the composition of essential oils changes from year to year, not only in terms of the number of individual components but also the number of identified components in the essential oil itself.

3.2. Antioxidant Effects of Essential Oils

Once absorbed into an animal's body, antioxidants play an important role in its physiological functions [78,79]. Researchers are now looking at the chemical nature and levels of natural antioxidants present in plant material as a part of an animal's diet, which is a natural source of antioxidants [80]. Flavonoids and other phenolic compounds are most commonly isolated in higher plants. Several flavonoids found in plants have protective effects against a variety of diseases, including cancer and allergy [81]. Medicinal plants have been extensively studied for the presence of natural antioxidants, but emphasis has been given to essential oils or their hexane, acetone, ethanol, and carbon dioxide extracts [82]. Aqueous extracts are more effective in scavenging hydroxyl radicals generated by the Fenton reaction and reducing oxygen consumption when initiated by metmyoglobin in cases other than methanol, ethanol, and acetone extracts [83,84].

Among the plants studied, the Lamiaceae family is frequently used as a source for extracting active components [85]. The chemical structure of extracts from the different tissues of these plants may correlate with their antioxidant activity; however, this relationship has not been explored in detail. Additionally, it is unclear to what extent the antioxidant activity of certain spices is attributed to essential oils. Essential oils from plants within the Lamiaceae family possess antioxidant properties, with a clear correlation between the inhibition of hydroperoxide formation and phenols such as thymol and eugenol [72,86].

Research on human and animal gut health is, nowadays, a major area of interest [87]. Any changes to gut health will certainly affect an animal's overall health and nutrient requirements. Hydrogen peroxide is constantly generated within all cell types, including gastric epithelial cells, and it has been implicated as a mediator of gastrointestinal injury [88]. Gastric epithelial monolayers, which are exposed to reactive oxygen species (ROS), may cause a significant decrease in trans-epithelial electrical resistance (TEER), which means the transport of ions across the epithelium [89]. When this transport is restricted, the electrical potential gradient increases across the epithelia. Different diseases are influenced by the intestinal barrier. To elucidate the passage routes and mechanisms involved in intestinal absorption and permeation, a widely used tool is the Ussing chamber [90]. By scavenging some free radicals, essential oils can help prevent many diseases, including brain dysfunction and immune system deterioration. Recent evidence suggests that free radicals are responsible for the development of these diseases [91–93]. Some essential

oils have also been shown to have anti-inflammatory properties, in addition to their ability to scavenge free radicals. Several mechanisms may account for essential oils' anti-inflammatory properties, including their antioxidant effect and their interactions with signaling cascades involving cytokines and regulatory transcription factors, as well as their effects on the expression of proinflammatory genes [93]. By controlling ROS release from neutrophils, thyme essential oil at 0.5 g/kg can also stimulate neutrophils, thus improving phagocytic activity [94], although the exact mechanism by which the essential oil of thyme affects antioxidant parameters is unclear. The cells may be using thyme essential oil's antioxidant properties, sparing their intracellular antioxidant systems [95].

Thymus vulgaris, *Origanum vulgare*, and *Rosmarinus officinalis*, along with their essential oils, provide poultry health benefits due to their direct antioxidant effects and the availability of phenolic terpenes such as carvacrol and thymol [95]. Lamiaceae oils influence the expression of genes, with an antioxidant response element (ARE) regulated by antioxidant enzymes [96]. The upregulation of these genes in the intestine seems to build a barrier against oxidative stress in the organism. In this way, botanical feed additives can improve the intestinal and general health of poultry and farm animals. The application of ARE-regulated genes can greatly increase intestinal barrier strength in food animals, which have a short life span because of their food production role. No studies have been conducted to determine the exact mechanism by which essential oils influence ARE-regulated genes. It can be only hypothesized that the different terpene compounds in essential oils can modify Keap1-like ECH-associating protein 1 (Keap1) at sensor-SH groups through chemical reactions. Keap1 is the protein in the cytosol with which the transcription factor Nrf2 is associated when the cells are protected against oxidative stress. Oxidative stress modifies Keap1 at redox-sensitive -SH groups, which leads to Nrf2 liberation and its nuclear translocation [97]. Subsequently, Nrf2 binds to the ARE promoter sequence of antioxidant enzymes [98]. In this way, Nrf2 coordinates cytoplasmic responses to oxidative stress. A diet rich in essential oils could explain an increase in TEER values in poultry. Accordingly, thyme essential oil strengthens the intestinal barrier against toxic feed-derived substances or endogenously produced toxic metabolites, reducing their absorption to improve the overall well-being of the animal [99].

4. Influence of Essential Oils on Productive Performance of Broiler Chickens

Global pressure to replace the use of antibiotics as growth promoters with safe feed additives in the broiler industry has led researchers to conduct a massive exploration into utilizing natural-substance-based additives. Essential oils are formed by dozens of complex mixture components that can be classified into a group of terpenoids (menthol, linalool, geraniol, borneol, α -terpineol) and a group of low molecular weight aliphatic hydrocarbons (thymol, carvacrol, eugenol, cinnamaldehyde). The advantageous effects of essential oils are associated with their role on many metabolic pathways, including lipid metabolism, stimulating digestive enzyme secretion and activity, acting as an antimicrobial, and enhancing the gut integrity of chickens, leading to improved broiler performance in general. Essential oils have proven to be important factors in protecting animals from various stressors, ensuring the optimal health and production characteristics of individual animals [100].

Many authors have stated that the addition of essential oils to the diet of broiler chickens had a positive effect on production characteristics [101–106]. Denli et al. [107] pointed out that the use of 60 mg/kg of thyme essential oil in the diet of quail chickens led to a statistically significant ($p < 0.05$) increase in growth, from 194.7 g (in the control treatment) to 206.3 g, and a statistically significant ($p < 0.05$) reduction in feed conversion ratios, from 3.40 kg/kg (in the control treatment) to 3.20 kg/kg [107]. In a study with the addition of a thyme extract to the diet of broiler chickens, Al-Kassie [108] found a statistically significant ($p < 0.05$) increase in the growth of broiler chickens during the fattening period (lasting 42 days), from 2546 g (in the control treatment) to 2617 and 2882 g in the treatments with the addition of 0.01 and 0.02 g/kg of thyme extract, respectively, in the diet of broiler

chickens. The same author [108] stated that at the end of fattening, the consumption of feed in the treatment with the addition of 0.01 and 0.02 g/kg of thyme extract was 4423 and 4612 g, respectively, while the value of this indicator in the control treatment was 4380 g; additionally, feed conversion was reduced by 1.72 kg/kg (control treatment) to 1.69 and 1.60 kg/kg in treatments with the addition of 0.01 and 0.02 g/kg of thyme extract, respectively, in the diet of chickens. In a study conducted by Toghyani et al. [109], by adding 5 and 10 g/kg of thyme powder to the diet of broiler chickens, the final body weights of 2079 and 1949 g were achieved, while the value established in control chickens of this indicator was 1956 g. In the treatment with the addition of antibiotics, the final body weight of broiler chickens of 2091 g was achieved. Regarding the average daily feed consumption for the entire fattening period, which lasted 42 days, no significant differences were observed between the experimental treatments, and the average values of this indicator ranged from 90.8 to 94.8 g. The same authors [109] stated that the feed conversion was reduced from 1.95 kg/kg (in the control treatment) to 1.86 and 1.90 kg/kg in the treatment with the addition of antibiotics or 5 g/kg of thyme in the form of powder in the diet of chickens; in the treatment with the addition of 10 g/kg of the same thyme powder, the value of this indicator was 2.03 kg/kg. Statistically significant differences at the end of the experiments are: control = thyme 10 g/kg < antibiotic = thyme 5 g/kg; therefore, dietary thyme supplementation was effective in improving chicken final weights at the lowest level of supplementation, but not at the highest level; the effect was concentration-dependant. Bozkurt et al. [110] pointed out that the addition of commercial preparations based on oregano led to an increase in the final body weight of broiler chickens by 4.44%, feed consumption by 2.95%, and a decrease in feed conversion by 1.53% compared to broiler chickens of the control treatment [110]. All these positive effects in enhancing poultry production performances can be the result of increased digestive enzyme secretions after the ingestion of essential oils. Previously mentioned trials have shown the positive effects of essential oils on nutrient utilization and poultry performance. However, it appears that the degree of response may be influenced by the level and type of essential oil used and the health status of the birds.

One of the most common mechanisms that explain the stimulating effect of essential oils on the production characteristics of broiler chickens is the impact of stabilizing feed hygiene, which affects the ecosystem of gastrointestinal microorganisms by reducing the number of unwanted bacteria [111–113]. Similarly, Kroismayr et al. [114] emphasized that essential oils and oleoresins affect the cecal microflora, which favorably affects the activity of desirable microorganisms in the gastrointestinal tract. By stabilizing intestinal health, animals are less exposed to toxins and undesirable products of microbiological activity, such as ammonia and biogenic amines. The formation of biogenic amines in the intestinal tract has been defined by researchers as undesirable, not only due to their toxicity but also since they are mainly formed by the decarboxylation of essential amino acids. For this reason, reducing microbiotic fermentation in the small intestines of individual animals can improve the availability of essential nutrients [114]. Windisch et al. [115] explained that essential oils have a beneficial effect on the organism in stressful situations and increase the availability and absorption of essential nutrients, which enables the more intensive growth of chickens and the achievement of maximum genetic potential. Reduction of microbiological activity also leads to the reduced production of volatile fatty acids, which affects the stabilization of intestinal pH, thus ensuring the optimal activity of digestive enzymes. Jamroz et al. [116] stated that the addition of plant extracts to the diet of broiler chickens affects the increased secretion of mucus covering the walls of the stomach and jejunum by creating a thin layer that has a protective role, reducing the possibility of the adhesion of undesirable microorganisms to epithelial cell mucosa.

High variability when it comes to the impact of essential oils on the production characteristics of broiler chickens can be due to the action of various internal (stress exposure, sex, age, etc.) and external factors, such as the physiological status of animals, breeding

methods, environment, infections, diseases, the composition of feed, and the content of active substances [55,117–119].

However, despite extensive research conducted over the last decade, it is still unclear which of these mechanisms is responsible for improving the production characteristics of broiler chickens or whether it is a combination of several mechanisms.

5. Influence of Essential Oils on Blood Biochemical Parameters in Broiler Chickens

5.1. Influence of Essential Oils on Enzymatic Activity in Blood Serum

The most important enzymes that play a role in digestion are amylase, protease, and lipase [120]. Amylase is the most important enzyme in the body for the digestion of carbohydrates, protease is for the digestion of proteins, and lipase breaks down fats into glycerol and fatty acids (the main sources of energy), as well as precursors of essential substances in the body [121]. The pancreas is a very important gland in the digestive system, and it plays a dual role in the body [122]. The exocrine function of the pancreas is the secretion of pancreatic juice, the main ingredients of which are enzymes for digestion (necessary for the processing of food in the intestines), which mixes with bile from the liver. It also secretes hormones such as insulin and glucagon into the blood, which act in other parts of the body; this is the endocrine function of the pancreas. The ratio of amylase, lipase, and protease in the juice of the pancreas depends on the age and diet of the chickens [122]. It is known that the secretion of pancreatic enzymes is regulated by acetylcholine (neurotransmitter) or cholecystokinin (hormone) [123]. Acetylcholine and cholecystokinin are released through nerves and intestinal cells when pancreatic enzymes are needed for digestion [124]. Some evidence suggests that exocrine pancreatic secretion is controlled by the vagus nerve [125]. However, other factors are thought to affect pancreatic enzymes or regulatory hormones and nerves. For example, dietary protein, medium-chain triacylglycerol, and amino acids have been reported to induce cholecystokinin secretion [125]. In addition, Boguśawska-Tryk [126] reported increased enzyme secretion in the pancreas when chickens were fed cellulose-fed feed. Platel and Srinivasan [127] have reported the stimulation of pancreatic enzyme secretion by plant extracts. It is believed that the activities of pancreatic enzymes increase the correlation with the weight of chickens, i.e., it is assumed that increasing the secretion of pancreatic enzymes will improve the performance of chickens [128].

As blood connects all tissues and organs in the body, it is assumed that every change in the body will be manifested by certain changes in the composition of the blood, which also applies to the activity of the pancreatic enzymes responsible for digestion [129,130]. Given the above facts, the blood of individual animals can play an important role in monitoring the health of the animal [131]. However, different genetic (sex, age, species) and paragenetic (diet, breeding conditions, welfare of individuals) factors can affect the value of the examined biochemical parameters and their interpretation, which should certainly be borne in mind. In some research, a positive effect of added essential oils on the activity of pancreatic enzymes in different animals was established [132,133]; however, according to the authors, this was based on a review of the available literature, a small number of studies examining the effect of phytobiotics in general on the activity of pancreatic enzymes in animal serum [115,134].

5.2. Influence of Essential Oils on Immunological Status of Broiler Chickens

Mucosal immunity is a very important part of humoral immunity, and it is immunoglobulin A (IgA) that is the effector of mucosal immunity [135]. They are found in the mucous areas of the digestive, respiratory and urogenital tracts, where they prevent the colonization of pathogens. They agglutinate antigens, neutralize viruses and bacterial toxins, and prevent the adhesion of pathogenic bacteria to the mucous membranes of epithelial cells. Plasma cells produce IgA antibodies, whose secretion through epithelial cells leads to the intestinal lumen [136]. Thus, IgA antibodies secreted by B-cells can be released into the gastrointestinal tract or circulation, increasing serum IgA concentrations [137].

Vaerman et al. [138] pointed out that approximately 30% of total plasma IgA originates from intestinal IgA synthesis.

Immunoglobulin G (IgG) occurs in an advanced stage of infection and is characterized by longer retention in the body, thus protecting it from renewed disease in a short time [139]. By proving the presence of this antibody, it can be shown that there was a previous disease or that immunization was performed.

More intensive broiler production has led to the need for new knowledge about the development and functioning of the immune system in broiler chickens. Broiler chickens are often exposed to various causes of stress and infectious diseases that can affect their innate and acquired immune response [100]. Inadequate immune response certainly affects the welfare of broiler chickens [140] and the ability to best express genetic potential, weakening the response to vaccine antigens, production effects, the emergence of various diseases, and, thus, increasing mortality during breeding, which ultimately affects the economic viability of production [1]. Physiological, genetic, and nutritional factors, as well as environmental factors, directly affect the immune system of broiler chickens, and the immunity of chickens can, therefore, be a sensitive indicator of farm management and broiler health [141].

Based on the review of available literature, some authors have pointed out the positive effect of essential oils in the diet on strengthening immunity in broiler chickens and piglets, increasing the secretion of immunoglobulins [117,142–144], but further research is needed to confirm the effects of essential oils on the animal immune response.

5.3. Influence of Essential Oils on Broiler Chickens' Blood Lipid Profile

Heart disease is a big problem in today's society, and it is directly related to increased levels of cholesterol in blood serum. Hypercholesterolemia and low levels of high-density lipoprotein (HDL) are often associated with endothelial dysfunction and inflammation, which are often accompanied by atherosclerosis [145]. Moreover, the concentration of cellular cholesterol is also associated with diseases such as Alzheimer's disease [146].

The effect of the addition of essential oils to the diet of broiler chickens in reducing the content of total cholesterol in the blood of chickens has been confirmed in various studies. Aghazadeh et al. [147] pointed out that the addition of thyme extract to the diet of broiler chickens reduced the content of triglycerides (TGs), TC, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and HDL in the following intervals, depending on the dose used in the supplements: 2.64–3.43%, 36.34–42.55%, 61.68–66.00%, 2.60–3.47%, and 3.28–20.63% compared to control treatment. The use of thyme extract in the diet of broiler chickens led to a statistically significant ($p < 0.05$) decrease in TG content, from 44.92 mg/dL (as determined in the blood serum of control broiler chickens) to 32.15 mg/dL and a reduction of LDL content from 47.03 mg/dL (as determined in the blood serum of control broiler chickens) to 32.15 mg/dL; there was also a statistically significant ($p < 0.05$) increase in HDL content from 45.56 mg/dL (as determined in the blood serum of control broiler chickens) to 51.22 mg/dL [147]. In a study conducted by Ghazalah and Ali [148], the addition of rosemary (0.5%, 1.0%, and 2.0%) to the diet of broiler chickens led to a statistically significant ($p < 0.05$) decrease in TC content in the blood serum of broiler chickens, from 124.70 mg/dL (as determined in the blood serum of control broiler chickens) to 95.83, 84.40, and 82.24 mg/dL, respectively. The same authors reported a decrease in LDL content from 54.00 mg/dL (as determined in the blood serum of control broiler chickens) to 38.00, 36.25, and 36.31 mg/dL, respectively [148].

Essential oils exhibit hypocholesterolemic effects in broiler chickens by inhibiting the most important enzymes involved in the synthesis of cholesterol and lipids, which significantly lowers cholesterol in the blood and edible tissues of broiler chickens but also significantly reduces abdominal fat in chicken carcasses. Abdulkarimi et al. [149] explained that one of the mechanisms of action of phytobiotics on cholesterol content may be the formation of insoluble saponin–cholesterol complexes in the gastrointestinal tract of chickens, whereby saponins present in phytobiotics inhibit the intestinal absorption of endogenous and exogenous cholesterol. Moreover, it is known that serum biochemical

indicators can display the nutrient's metabolism and the body's physiological state. In poultry production, dietary supplementation of essential oils improves the serum lipid profile by reducing cholesterol and LDL-C concentrations in serum. The active substances in essential oil have been reported to possess cholesterol-lowering effects because of 3-hydroxy-3-methyl glutaryl-CoA reductase protein expression, leading to decreased total cholesterol and LDL-C concentrations. Essential oils also exhibit hypocholesterolemic properties through the activities of HMG-CoA reductase and cholesterol-7 hydroxylase fatty acid synthase and by 6-amino nicotinamide inhibiting the activity of the pentose phosphate pathway [150]. As mentioned before, one of the possible mechanisms of lipid alteration due to the presence of dietary essential oil in the gut is the inhibition of HMG-CoA reductase activity in the liver [151].

Contrary to the above, there are opinions that essential oils do not have a positive effect on reducing the content of total cholesterol in the blood of broiler chickens. In a study by Toghyani et al. [109], thyme powder added to the diet of broiler chickens did not lead to a decrease in TG and TC contents or a decrease in LDL concentration. In a study by Bölükbaşı et al. [152], the addition of 100 and 200 mg/kg of thyme essential oil to the diet of broiler chickens led to a statistically significant ($p < 0.01$) increase in TG content, from 91 mg/dL in the blood serum of control chickens to 122 and 135 mg/dL, respectively. There was also an increase in LDL content from 52 mg/dL in the blood serum of control chickens to 61.50 and 62.50 mg/dL, respectively, in the blood serum of broiler chickens with thyme essential oil added to their diet. The same authors reported a statistically significant increase in HDL content from 91 mg/dL in the blood serum of control chickens to 110 and 97 mg/dL, respectively, in the blood serum of broiler chickens fed experimental diets [152]. Similar conclusions were reached by Saleh et al. [153] in a study with the addition of thyme and ginger to the diet of broiler chickens. Demir et al. [154] stated that the addition of commercial preparations based on thyme and oregano did not affect TG and TC contents in the blood of broiler chickens; a similar outcome was found for the addition of antibiotics, confirmed by the study of Popović et al. [100].

These blood parameters are crucial when it comes to the health of broiler chickens, and they are often responsible for detecting various health disorders during intensive breeding; they are also important in terms of the quality and safety of chicken meat, indicating the importance of such natural supplements in broiler chicken diets.

6. Influence of Essential Oils on Liver Histo-Morphological Changes and Intestinal Villi Morphology

The chicken intestine is a complex digestive organ composed primarily of three parts of the small intestine—duodenum, jejunum, and ileum—which come into contact with a large number of intestinal microbiota [155]. The mucosa in which nutrients are absorbed consists of epithelial cells that rely on specialized mucosal structures called “intestinal villi” and “crypts” [156]. The intestinal villi are a repetitive formation that represents a protrusion in the intestinal lumen, while the crypts, located on each side of the intestinal villi, represent the invagination of the epithelium. In chickens, the development of intestinal villi and crypts is seen in the period after hatching [157]. The intestinal villi are considered to be a specialized structure for increasing the absorption surface. Therefore, in many studies, the height of the intestinal villi (Vh) is measured histologically to indicate an increased area for nutrient absorption [158].

The intestinal epithelium acts as a natural barrier against pathogenic bacteria and toxic substances found in the intestinal lumen [159]. Stressors, pathogens, and chemicals, among others, cause disturbances in the normal microflora or intestinal epithelium that can alter the permeability of this natural barrier, facilitating the invasion of pathogens and harmful substances and modifying their metabolism and ability to digest and absorb nutrients, leading to chronic inflammatory processes in the intestinal mucosa. Accordingly, there is a shortening of the intestinal villi, an increase in cell turnover, and a decrease in the activity of the digestive tract and absorption [160–162].

Since the absorption of nutrients depends on the process that takes place in the intestinal mucosa, essential oils are widely used to improve the production characteristics and efficiency of the intestinal mucosa. The mechanism of action of essential oils can be explained by the production of antimicrobial substances and organic acids, the protection of intestinal villi, and the absorption of surfaces from toxins produced by undesirable pathogens, as well as the stimulation of the immune system. On the other hand, the action of essential oils is based on reducing the growth of many pathogenic or non-pathogenic intestinal bacteria by lowering the pH [163], which is the result of increased lactic acid levels in the intestine. Some bacteria can recognize such binding sites in the molecules when they are on the surface of the mucosa, and the intestinal colonization of pathogenic bacteria is thus reduced [164]. Therefore, there is a lower frequency of infectious processes, and the functions of secretion, digestion, and absorption of nutrients can be adequately performed through the mucosa.

Physiological mechanisms associated with plant-derived supplements alleviating clinical and subclinical symptoms of different infections and improved growth are limited. Bioactive compounds, such as carvacrol, cinnamaldehyde, and capsicum oleoresin, have been noted to have an effect on stimulating the production and secretion of mucin in the intestine, thereby possibly impairing the adhesion of pathogens and having a positive effect on the gut of the chicken. Further benefits include influences on nutrient digestibility, nutrient absorption, and intestinal morphology and a stabilizing effect on the intestinal microbiota. Reisinger et al. [165] evaluated the effects on performance in broilers given a phyto-genic feed additive containing essential oils from oregano, anise, and citrus peel and on intestinal morphology during coccidial vaccine exposure. The results showed that chickens fed the essential oil supplement had 12% deeper crypts than control chickens, while chickens on the coccidial vaccine had an 11% reduction in crypt depth [165]. The morphology and functionality of the different regions of the intestinal tract seem to be a flexible system that is able to adapt to the needs of the organism. However, little information is available on how bioactive compounds affect gastrointestinal morphology and functionality. Jamroz et al. [116] observed qualitative increases in the number of goblet cells and mucin secretion at the surface of the villi of the jejunum when feeding broilers a mixture of 5, 3, and 2 mg/kg of carvacrol, cinnamaldehyde, and capsicum oleoresin. Additionally, Reisinger et al. [165] revealed that dietary supplementation with essential oils had the most notable effects on chicken mid-ileum morphology, causing an increase in crypt depth (but not enterocyte turnover rate) as well as an increase in the total number of goblet cells (by 30% for naturally exposed chickens, with pen-mates given the 1 × coccidial vaccine dosage).

Numerous positive effects of essential oils on the intestinal mucosa have been noted, including a significant increase in intestinal villi in three segments of the small intestine; this contributes to increased nutrient absorption, better digestibility of nutrients, and, thus, improved production characteristics.

7. Influence of Essential Oils on Broiler Chickens' Meat Quality

After the ban on the use of antibiotics in animal nutrition, phytobiotics have proven to be a good alternative solution; hence, they are successfully used in the diet of broiler chickens to improve the nutritional, technical, and sensory qualities of chicken meat [14,166]. The positive influence of medicinal plants and essential oils on the nutritional and technical parameters of chicken meat quality has been confirmed in many studies [17,55,167–170].

In a study conducted by Young et al. [171], the addition of oregano to the diet of broiler chickens did not affect the pH value of chicken meat but contributed to an increase in the proportion of yellow (b^*) in breast meat and thigh meat, from 2.9 to 4.1 and 3.0 to 4.7, respectively. Lipiński et al. [172] have investigated the effect of herbal feed additives on the growth performance, carcass characteristics, and meat quality of broiler chickens fed low-energy diets. They have shown that increased meat acidity (measured 15 min postmortem) was higher in the groups fed dietary herbal additives. It is assumed that

herbal feed additives have contributed to a highly significant increase in the water-holding capacity (WHC) of meat and natural drip loss, an increase in pH₁₅, an increase in color lightness, a decrease in redness, an increase in the fat content of the meat, and a decrease in malondialdehyde (MDA) and dietary energy concentrations. The performance of broiler chickens fed different levels of thyme, adiantum, and rosemary and their combination was investigated by Tayeb et al. [173]. Their findings indicated that different levels of thyme, adiantum, and rosemary and their combination had an effect on broiler meat color. The different levels of medicinal plants had no significant effect on the lightness, redness, and yellowness of meat color in broilers compared to the control group. The results were in agreement with the findings of a study that reported that dietary quercetin and methoxylated quercetin extracted from onion in broiler diets had no significant effect on broiler meat color [174,175]. Regarding the antioxidant potential and meat quality of Japanese quail fed with the dietary essential oil of thyme, an investigation was conducted by Onel and Aksu [176]. Research has shown that the inclusion of thyme essential oil supplementation into the diets of quails in high stocking density had a significant effect on the pH and color of the brisket [176]. Choe et al. [177] investigated the influence of thyme and star anise essential oils on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color, and relative organ weight after oral challenge with *Clostridium perfringens* in broilers. Besides numerous positive effects, the results of these studies have revealed no significant differences in broiler breast pH and meat color between dietary essential oil and control treatments. Furthermore, Alfaig and Angelovi [178] pointed out that the addition of thyme essential oil has a small effect on the hardness of chicken meat. Hong et al. [179] found that the addition of oregano essential oil to the diet of broiler chickens had a positive effect on the hardness of the breast meat, while the appearance, aroma, and juiciness of the meat did not differ between individual treatments. The same authors stated that the meat of chicken drumsticks fed with oregano essential oil was much juicier compared to the meat of drumsticks of control broiler chickens and chickens to which antibiotics had been added; they also stated that oregano essential oil significantly improved the appearance of drumstick meat compared to antibiotic-fed chicken meat [179]. The positive effect of essential oils on the sensory properties of chicken meat has been proven in many other studies [101,166,180,181].

Despite a large number of studies in which the positive effect of essential oils added to chicken diets on meat quality has been proven, the mechanism of action of these components is still unknown. Ri et al. [182] stated that the addition of oregano powder to the diet of broiler chickens did not lead to changes in the quality of chicken meat, which, the authors explained, was influenced by various factors: plant species, the composition of the chicken's diet, the concentration of added phytobiotics, environmental conditions, and interactions with feed components. It should be borne in mind that the results of fattening are influenced by factors such as genotype, health, and hygiene conditions in the production facility; hence, the positive impact of phytobiotics on selected quality parameters cannot always be manifested [182]. A study by Pavelková et al. [183] noted that the addition of oregano did not positively affect the sensory parameters of meat quality, which, the authors explain, was the subjective assessment of the panelists since the specific aroma of oregano is not an easily acceptable dietary supplement.

Additionally, bioactive compounds derived from essential oils have properties that benefit not only the animals fed with them but might indirectly impact the consumers of the meat. Oregano essential oil has antioxidant and antimicrobial properties due to its phenolic components, mainly thymol and carvacrol. The active components of oregano essential oil are potent antimicrobials affecting populations such as *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, protozoa, fungi, *Ruminococcus fibrisolvens*, and *Fibrobacter succinogenes*; this change in populations modifies the gut environment, which is fundamental in the conversion of dietary nutrients to muscle tissue. Specifically, there is evidence that carvacrol potentially decreases acetate concentrations and increases propionate and butyrate [184]. Both are volatile fatty acid precursors of muscle and fat components in the

animal, which could be one of the explanations for how bioactive components from dietary essential oils can improve the quality of meat.

8. Conclusions

Results presented in this review have shown that the active components present in the essential oils of thyme, oregano, and rosemary stimulate the activity of beneficial bacteria, thus contributing to a balanced microflora, i.e., an effective prerequisite for protection against pathogenic microorganisms. Increasing the number of beneficial bacteria not only reduces the number of available substrates for pathogenic microorganisms but also stabilizes intestinal pH, thus ensuring the optimal activity of pancreatic enzymes, which further leads to the improved digestibility of nutrients and, thus, improved production characteristics. Moreover, it can be concluded that the biologically active components present in the used essential oils improve the intestinal morphology and increase the height of the intestinal villi and the depth of the crypts, which also contribute to improving the production characteristics of chickens.

Therefore, a balanced diet for broiler chickens, with the optimal composition and content of a mixture of essential oils of thyme, oregano, and rosemary, can achieve positive results (i.e., economical fattening) and produce broiler chickens that have good yields, more meat on the carcass, and significantly improved meat quality.

The chicken meat obtained in this way will enable producers to sell to the demanding markets of the EU and beyond, reflecting the scientific and practical significance of this paper.

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