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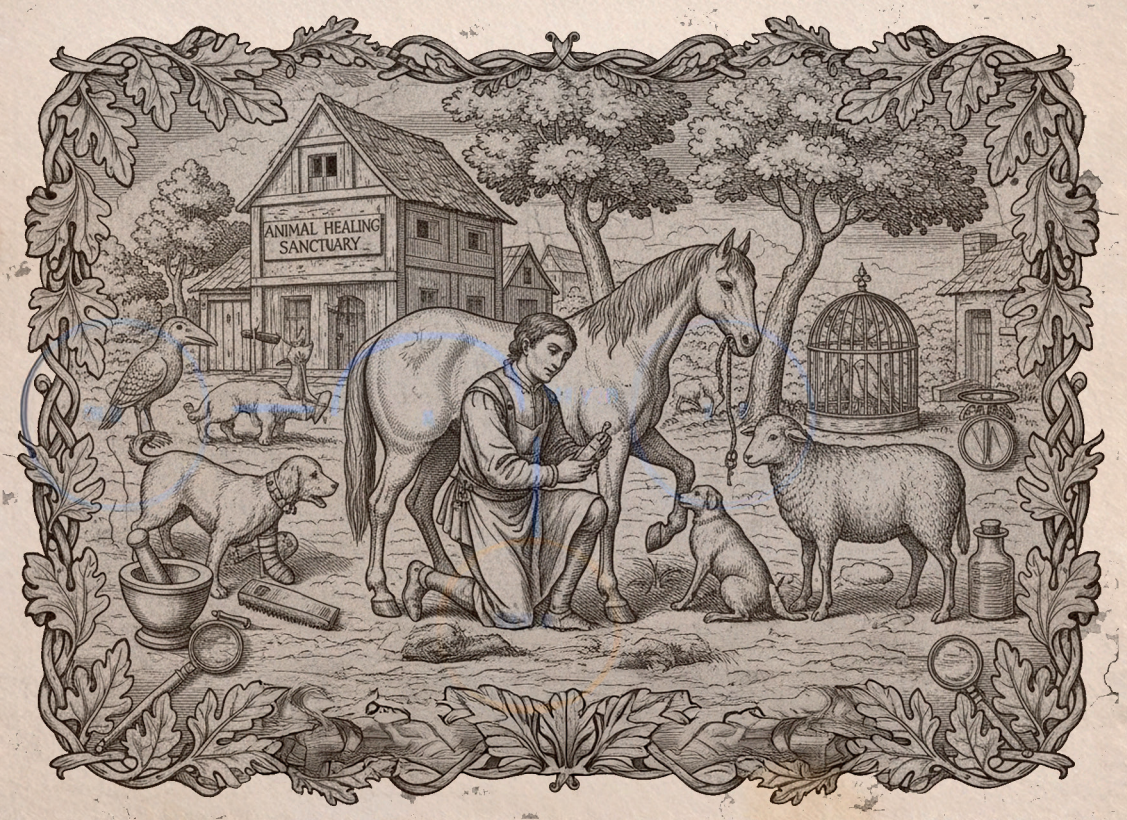
EDITOR **PROF. DR. KEZBAN ŞAHNA**



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CONTENTS

Chapter 1

MOLECULAR INSİGHTS INTO THE ANTICANCER POTENTIAL OF GALBANIC ACID

Ayşe AYDOĞAN [1]

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INTRODUCTION

Despite the advances in multidisciplinary treatments; cancer is still one of the most intricate and difficult diseases to control in human health due to the multi-etiologic, molecular heterogeneity and dynamic microenvironment interaction with host. Although tremendous progress has been made in early detection and treatment, cancer remains one of the most prevalent causes of morbidity and mortality worldwide. The complex biologic nature of carcinogenesis, including genetic mutation, epigenetic modification, disruption in cell-cycle control, immune escape mechanisms and angiogenesis/metastasis is a major challenge to developing universally efficacious therapies. As a result, new treatment strategies to exploit many different hallmarks of cancer simultaneously with reduced toxicity and resistance remain required.

Natural products have gained great attention in the past several years as excellent chemical entities for discovery of bioactive compounds that can be used for cancer prevention and treatment. Introduction Nature has historically been an important source for drug discovery and many cancer chemotherapeutics have originated from, or based on, plant secondary metabolites. These agents commonly demonstrate pleiotropic biological effects which in turn confer the ability to regulate various molecular signaling pathways associated with tumorigenesis, tumor progression and therapeutic resistance. Besides their direct cytotoxic effects against cancer cells, numerous natural products have antioxidant, anti-inflammatory and immunomodulatory activities which add to their general antitumor potential.

Contrary to the heterogeneous groups of plant metabolites, sesquiterpene coumarins belong to the class of plant bioactive molecules relating a broad spectrum of potential therapeutic activities. Galbanic acid (GA) a natural sesquiterpene coumarin which has been found mainly in plants of the Ferula genus is one of the compounds on which more attention has been focused in recent years by researchers. Experimental evidence has been accumulated for galbanic acid to exert diverse biological activities such as antioxidant, cytoprotective, antimicrobial, antiviral and anticancer activities. It is worthy of note that, galbanic acid has previously been documented in the literature to influence the multidrug resistance processes including efflux transporters inhibition which will lead to enhanced intracellular bioavailability and effectiveness of co-administered drugs.

In consideration of the increasing number of reports demonstrating the multi-faceted biological activities of galbanic acid, a full understanding about its chemical characterization, mode(s) of action and therapeutic potential needs to be established. The main purpose of this book chapter is to offer a

global comprehensive overview of what we know about carcinogenesis and the importance on it of galbanic acid. In particular, the chapter examines how galbanic acid can influence major signaling pathways linked to oxidative stress, apoptosis, drug resistance and carcinogenesis in general and speculate on its potential usage as supportive treatment or adjuvant for the current cancer therapeutics. The integration of basic cancer biology with recent findings on galbanic acid in this chapter aims to support the already available outcomes relating this natural compound as a potential agent for control of cancer.

1. Carcinogenesis

Cancer is a neoplas-tic disease in which there is excess and uncontrolled proliferation of cells due to insufficient levels of apoptosis (Alberts et al., 2002). The word "cancer" is derived from the Greek karkinos, meaning crab, and "genesis" from genesis or creation. Out of the fusion of these two terms arose the notion of carcinogenesis, that is, the development of cancer (Vogelstein & Kinzler, 2002). Carcinogenesis is developed by gradual accumulation of mutations in processes that are fundamental to life, like cell proliferation and survival, growth, differentiation. This process is multifactorial and includes activation of proto-oncogenes, inactivation of tumor suppressor genes resulting to malignant transformation (Vogelstein & Kinzler, 2002).

These aberrant cells also evade the normal apoptotic stimuli which limit the population of certain cell types and continue to proliferate uncontrollably, creating masses of abnormal cells referred to as tumors or neoplasm (Weinberg 2007). Tumors are classified as benign and malignant. Benign noncancerous tumors are growths that do not spread metastasize, tend not to destroy surrounding tissue, and do not usually recur after they are removed surgically. Malignant tumors, on the other hand, are comprised of cells that grow rapidly and abnormally. They're also capable of invading tissues, creating structural destruction and metastasizing to other areas. Cancer cells may break away from the original tumor and travel to other parts of the body through the blood or lymph metastasis where new tumors form (Vogelstein & Kinzler, 2002). Thus, to be recognised as a cancer, a tumor must display malignant features such as uncontrolled expansion and have the capability of local invasion and distant spread (Vogelstein & Kinzler, 2002).

Among them, the most notable hallmark abilities of cancer cells are uncontrolled proliferation, insensitivity to anti-growth signals from other cell types, evasion of apotosis, induction of angiogenesis and tissue invasion (Akbulut, 2005). Because the epidemiological burden of cancer is so great, studies at the molecular level are essential to increase understanding, improve diagnosis and facilitate treatment. A wide array of tumors in a different cancer lineages have been developed and efforts to search for sensitivities of those

tumorigenic cell lines are underway. Researchers attempt to identify what metabolic pathways have broken down in normal cellular metabolism, how such disruptions drive disease pathogenesis, and identify therapies that can be applied (Alberts et al., 2002). Of these, apoptotic signalling cascades have been the focus of interest (Ayhan 2013).

Apoptosis, the programmatic process of cell death, is an important physiological process for normal cellular turnover and tissue homeostasis (Mak, 2003). While some genetic changes occur during carcinogenesis, others are heritable while still others are the result of exogenic factors. These mutations can be loss of chromosome, instability of the genome, chromosomal rearrangements or insertion of foreign DNA into human chromosomes or loci which are often viral in origin (Fışkın, 2002). There are also environmental risk factors that contribute to the development of cancer such as diet, radiation exposure and prolonged exposure in the workplace to cancer facilitating substances. Notorious examples include the relationship of benzene to leukemia and asbestos or arsenic exposure to lung cancer (Bilir, 2007).

The incidence of cancer is still increasing throughout the world, and it is still one of the major causes of death around the globe following cardiovascular disorders (Kaur et al., 2018). It is estimated that, by The World Health Organization (WHO) 2030, cancer will be the source of death for 63% of non-communicable diseases. Lung, liver, stomach, breast and colorectal cancers are the deadliest cancer types (Sankari et al., 2012). Breast and lung cancer are the most commonly diagnosed cancers in females; prostate, lung and colorectal cancer are the most common in males (Miller et al., 2016).

In addition to traditional cancer treatment methods of chemotherapy and radiotherapy, natural components have become more popular in current science due to cancer prevention and therapy. Experimental and clinical research during the past few decades has revealed that many of the bioactive compounds present in plants polyphenols, flavonoids, phenolic acids possess various biological activities related to cancer development. The interest in these compounds is increased since they inhibit the proliferation of tumor cells, at the same time show antioxidant and anti-inflammatory effects. There is increasing evidence that natural products can target critical key molecular pathways in carcinogenesis via regulation of both genetic and epigenetic events. Through these interactions, they can trigger apoptosis, halt the cell cycle, inhibit angiogenic signals and modulate the immune response in the tumor microenvironment.

The capability to hit several hallmarks of cancer simultaneously has thus placed bioactive compounds of natural origin as valuable candidates for adjunct and supportive cancer treatments. Among them, galbanic acid,

a sesquiterpene coumarin obtained from species of the genus Ferula has received increasing interest owing to its wide range of bioactivities. It has been suggested that galbanic acid is a cytotoxic molecule with antioxidant and anti-cytotoxic, multidrug resistance modulatory, and anticancer actions which seem to implicate Galbanic acid in a variety of carcinogenesis-promoting mechanisms. Its regulation of cell proliferation, apoptotic signaling pathways, oxidative stress responses and multidrug resistance (MDR) mechanisms demonstrate the potential of galbanic acid as an adjuvant in cancer treatment. Therefore, profound clarity on the chemical characteristic, activity and therapeutic potential of galbanic acid against cancer from the point of cancer biology is required as it may result in discovery natural compound-based innovative strategies during contemporary time period in oncology.

2. Chemical Structure and Physicochemical Properties of Galbanic Acid

Galbanic acid shares a structural feature of the sesquiterpene coumarins class which consists of a sesquiterpene side chain reversibly echopped on to a coumarin moiety (Nazari & Iranshahi, 2011). It has a molecular formula of $C_{24}H_{30}O_5$ with Listed molecular weight at approximately 398.49 g/mol (Wang et al., 2020) The coumarin ring in the chemical structure of galbanic acid plays an essential role in contributing significant biological activity and more so, enhances its ability to interact with cellular signal pathway (Cha et al., 2011) Presence of the sesquiterpene side chain imparts strong lipophilicity properties to Galbanic acid promoting it's crossing through biological membrane via passive diffusion process (Kasaian et al., 2013). Galbanic acid is less soluble in water but highly soluble in organic solvents including ethanol, methanol, chloroform and DMSO from the physicochemical point of view. Such lipophilic properties directly affect galbanic acid's pharmacokinetic behavior thus potentially improving its interaction with intracellular targets proteins (Kasaian et al., 2013; Wang et al., 2020). İn conclusion the coumarin-based structure, lipophilicity and membrane permeability of galbanic acid were recognized as a crucial chemical property contributing to the molecular mechanism underpinning the anti-cancer activities of this compound.

3. Sources of Galbanic Acid

Galbanic acid is a natural sesquiterpene-coumarin compound from species in the Ferula genus (Apiaceae; Umbelliferae) reported by Kasaian et al. (2013). This product is most prevalent in roots and resins of Ferula galbaniflua, Ferula assa-foetida, and the related giant fennel (Ferula szowitsiana) but also all well known smaller ornamental plants as well. Chemical structure of galbanic acid belongs to a prenylated coumarin derivatives; the special chemical structure determines the molecule's biological activities (Kasaian et al., 2013).

4. Pharmacological and Therapeutic Potential of Galbanic Acid

AnimaI studies Galbanic acid has recently demonstrated to ameliorate lead acetate-induced reproductive toxicity through modulating oxidative stress markers and histopathological injury was suppressed. These results indicate that galbanic acid can have a protective effect under conditions of cell stress and has several pharmacological functions (Gharibshahi et al., 2025). Furthermore, galbanic acid has been identified to modulate multidrug resistance (MDR). Galbanic acid decreases drug efflux from the cell membrane by suppression of P-glycoprotein (P-gp) activity, and results in elevated intracellular concentrations of a wide range of pharmacological agents. This characteristic makes galbanic acid to be seen as not only a gterapeutic molecule in direct, but also an adjunctive nucleon us molecular aspect with the current drugs (Hanafi-Bojd et al., 2011). The antioxidant and cytoprotective activity of galbanic acid under oxistress conditions has been suggested. In animal studies, galbanic acid has been observed to modulate oxidative stress parameters in relation to heavy metal exposure and prevent tissue damage. These findings indicate that galbanic acid could be used as a protective phytochemical against toxic compounds (Gharibshahi et al., 2025). In addition, the literature is replete with antibacterial and antimicrobial properties of galbanic acid. Galbanic acid is not a strong antibacterial drug, but it can improve the antibacterial activity of antibiotics through reducing resistance in microorganisms. This effect is especially due to the suppression of bacterial drug pumps, indicating a synergistic relation between galbanic acid and other drugs (Bazzaz et al., 2010). Besides these effects, it is mentioned that galbanic acid may also possess antiviral (Mossa et al., 2013), anticoagulant, and antileishmanial activities. This broad spectrum of biological activities implies the potential use of galbanic acid ranging from traditional medicine to modern pharmacology (Mossa et al., 2013). The potential anticancer effect of galbanic acid has been suggested in recent cellular studies (Oh et al., 2015).

5. Anticancer Mechanisms of Galbanic Acid

Galbanic acid, a natural sesquiterpene coumarin compound, may be useful in combating cancer cells through several molecular mechanisms. Such mechanisms also result in alterations of intracellular signal transduction, as well as microenvironmental conditions outside the cell.

Apoptosis Induction: GBA induces cell death, in NSCLC models in particular, through the regulation of apototic proteins. Under these conditions, GBA favor events such as PARP degradation, caspase-9 activation and the increase of pro-apoptotic protein Bax. It as well decreases the quantities of anti-apoptotic proteins such as Bcl-2, Bcl-xL, and Mcl-1 present in the cell leading to greater apoptosis. There are models for which this phenomenon

has been shown and a considerable number of apoptotic cells were found to be induced GBA-treatment (Oh et al., 2015).

Induction of Death Receptor Pathways and inhibition of MDR: Galbanic acid induces death receptor 5 (DR5) pathway while significantly abrogating the expression of multidrug resistance protein 1 (MDR1) especially in resistant cancers. The activation of significant apoptotic enzymes such as caspase-8 and 9 is mediated by signaling through DR5. When MDR1 is knocked down, export of chemo drugs from the cell is blocked and intracellular drug levels are elevated resulting in higher drug effectiveness. This pathway directly promotes apoptosis and sensitizes to chemotherapy (Kim et al., 2019). Galbanic acid possesses cell cycle-arrest and anti-proliferative activities in cancer cells. The blockade of cell cycle leads to arrest in a specific cell cycle phase such as G2/M phase, which restricts the multiplication process (Kim et al., 2011).

PI3K/Akt/mTOR Signaling Pathway: A few studies reported that galbanic acid modulated cancer cells by inhibiting cell survival pathway including PI3K/Akt/mTOR. PI3K/Akt/mTOR is one of the most important pathways for cell growth and survival across many cancer origins. Being an inhibitor of this signaling pathway, galbanic acid is also able to elevate the expression of the tumor suppressor PTEN thus restraining the cell proliferative capabilities. This has been experimentally validated, notably in glioblastoma cell models. In the same study, reduction in activity of enzymes such as MMP (MMP9 and particularly MMP2) for conservation of the migratory and invasive potential of cancer cells due to galbanic acid have also been reported (Shahcheraghi et al., 2021).

Anti-angiogenesis Efficacy: Galbanic acid has been reported to exert an inhibitory effect on angiogenesis. Has been reported to inhibit tumor growth, potentially by blocking vascular cell proliferation driven by VEGF ligand-mediated cues (Kim et al., 2011).

6. Anticancer Activities of Galbanic Acid

Anticancer Activities of Galbanic Acid in Breast Cancer: The antiproliferative activities of galbanic acid in breast cancer were tested on estrogen receptor negative (ER-) cell line MDA-MB-231. It was a remarkable finding that the inhibition of cell viability in this cell line could be achieved dose and time dependently by galbanic acid, which showed a marked anticlonogenic effect. Similarly, it was demonstrated that MDA-MB-231 breast cancer cells treated with galbanic acid lead to apoptosis. Galbanic acid treatment increased Bax expression and reduced Bcl-2 expression, a change was shown to be mediated by a mitochondrion-mediated cytotoxicity pathway (Sajjadi et al., 2019).

An other prominent effect of galbanic acid on breast cancer are the MDR molecular mechanisms. A study by Hanafi-Bojd et al. showed that inhibition of drug efflux from P-glycoprotein (P-gp) by galbanic acid. This research demonstrated that-cell a galbanic acid-induced inhibition of -Rhodamine-123 efflux in MCF-7/Adr cells, which resulted in intracellular drug accumulation. These results imply that galbanic acid might resensitize drug-resistant breast cancer cells to chemotherapy (Hanafi-Bojd et al., 2011). It has previously been shown that galbanic acid could modulate the cellular redox status of breast cancer cells. It has been demonstrated that galbanic acid application in ER-type breast cancer cells, can modulate cellular redox balance and induced alterations related to antioxidant response (Sajjadi et al., 2019). Taking the present findings together with those from current literature, it seems that galbanic acid inhibits breast cancer-cell proliferation and induces apoptosis through avoiding multidrug resistance. Potential effects on cell proliferation and apoptosis Although these effects have been shown only in in vitro cell culture models, there is no such information from in vivo or clinical trials (Hanafi-Bojd et al., 2011; Sajjadi et al., 2019).

Anticancer Activities of Galbanic Acid in Colon Cancer: The majority of the researches have investigated the role of galbanic acid on colon cancer based on cell cultures and animal models, particularly those about its antiproliferative and tumor angiogenic roles. It has been shown that galbanic acid carried by PLA-PEG nanoparticles shows a greater cytotoxic response and a more marked ability to decrease cell viability in C26 colon carcinoma cell line than the free form. The followed study showed that galbanic acid-loaded NPs could inhibit tumor growth and decrease the density of CD34-positive vessels in an in vivo colon tumor model, indicating a potential anti-angiogenic outcome in colon cancer for galbanic acid (Afsharzadeh et al., 2019). C26 colon cancer cancer cell death was increased when galbanic acid was transported to the cells by PLGA nanoparticles loaded in human mesenchymal stem cells. These findings indicate that combining galbanic acid with drug delivery systems may have improved therapeutic potential in colon cancer management (Ebrahimian et al., 2022). Furthermore, reported studies for combined therapy assessments of Galbanic acid in combination with nano micelle curcumin was found to be a more potent inhibitor of cell proliferation in C26 and Caco-2 colon cancer cells than trials with individual applications, producing a synergistic anticancer effect. Thereby this report indicates that galbanic acid, when combined with other natural or chemothrapeutic agents for colon cancer might potentiate treatment efficiency (Jafari et al., 2019).

Anticancer Activities of Galbanic Acid in Lung Cancer: Less is known about galbanic acid in lung cancer, where its role is indirectly debated through the MDR effect, not eliciting cytoxicity. P-glycoprotein (P-gp) associated

drug efflux is considered one of the major reasons for chemotherapy failure in a variety of solid tumors such as lung cancer. Galbanic acid has been demonstrated to prevent the P-glycoprotein (P-gp) mediated drug efflux, inhibit Rhodamine-123 efflux in several cancer cellular lines and enhance the intracellular drug accumulation. It indicates that galbanic acid may be a candidate chemosensitizing substance for overcoming the common problem of lung cancer resistance to chemotherapy (Hanafi-Bojd et al., 2011).

Anticancer Activities of Galbanic Acid in Lymphoma Cancer: It has been shown that galbanic acid, obtained from F. szowitsiana, possesses cytotoxic activity against human T-cell leukemia/lymphoma (MT-2) cells and this effect is enhanced in hypoxia. This result indicates that galbanic acid could also be active in hematological cancer models (Goudarzi et al., 2022).

Conclusion

Cancer is a disease that has heterogeneity at multiple levels and is characterized by an intricate network of genetic, epigenetic, and microenvironmental factors leading to tumor initiation and progression tumorigenesis, metastasis development, as well as therapeutic resistance. Although there have been great achievements in traditional cancer treatments, treatment-associated toxicity, multidrug resistance and disease recurrence still present considerable limitations, supporting the development of complementary strategies that can simultaneously target several different hallmarks of cancer. In such context, natural bioactive compounds have been proposed as potential candidates for these indications on the count of the pleiotropy of its actions and good tolerability profile.

The scope of this chapter has been to discuss the major biological events in carcinogensis and underscored the increasing importance of plant-derived chemicals in their control. Among these com-pounds, galbanic acid, a sesquiterpene coumarin isolated from the genus Ferula has been considered mainly due to its wide range of biological activities. Continued pre-clinical studies The accumulating body of experimental data has shown that Gallic acid could modulate oxidative stress, induce apoptosis, judge proliferation and even intercept important drug resistance mechanisms as active efflux membrane proteins leading to the blockade of P-glycoprotein transporter. These mechanisms show that galbanic acid has the potential to improve the efficacy of chemo agents and become a synergistic molecule, but not as an individual cytotoxic medicine.

Notably, in vitro and in vivo studies have demonstrated that galbanic acid has cytoprotective activity against this stress, with an ability to discriminate between pathologic events that trigger cancer development. The capacity of

targeting different molecular substrates allows galbanic acid to be considered as a promiscuous compound with putative applications in combination therapy protocols for the prevention or counteraction of therapeutic resistance and treatment response amelioration. However, it must be appreciated that the majority of existing data come from in vitro an imal models, while detailed clinical studies are scanty.

In summary, galbanic acid appears to be an attractive potion of both folk remedies and modern pharmacology. Further investigation of the molecular mechanisms, pharmacokinetics, security profile and clinical efficacy is necessary to better understand its therapeutic value. Then, future investigations including galbanic acid in refined combinations will provide more powerful, personalized and sustainable lifestyle based anticancer therapies.

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Chapter 2

An Overview of Livestock Production Systems in Turkey with Emphasis on Malatya Province

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Introduction

Animal husbandry plays a significant role in the economies of almost all countries around the world. Livestock farming has made important contributions to the social and economic development of societies. Throughout history, animals have been one of the most important assets and sources of wealth for humans. With the rise in the cultural and educational levels of societies and industrialization, animal husbandry has become an industry today. This has led to significant increases in the yields obtained from animals (Oğan, 2015).

Livestock farming is a multifaceted agricultural production activity that not only meets human needs (meat, milk, eggs, etc.) but also ensures the continuity of plant production, converts agricultural and industrial waste and other materials that cannot be otherwise utilized into products of economic value, and creates employment opportunities by generating labor in this field. In the developing world, the need for animal products, which are important and essential for human nutrition, is increasing in response to rapid population growth. For balanced and adequate nutrition, there are proteins that cannot be produced by the human body and must be obtained from external sources. Animal-based proteins are at the top of this list (Aysöndü & Koçyiğit, 2016). For an adult to be able to eat a balanced diet, they need to consume 2800-3000 calories and 75-80 g of protein per day. Of this protein, 30-35 g must be of animal origin (Oğan, 2015).

This study compares the current state of cattle and sheep farming in Turkey and Malatya, examines the problems in this area, and proposes some solutions for the development of cattle and sheep farming.

Malatya Province

Malatya is located in the Upper Euphrates Basin of the Eastern Anatolia Region and at the southwestern end of the Adıyaman, Malatya, Elazığ, Bingöl, Muş, and Van depression area. It is surrounded by the provinces of Elazığ and Diyarbakır to the east, Adıyaman to the south, Kahramanmaraş to the west, and Sivas and Erzincan to the north. The province has an area of 12 313 km^2 and lies between 35°54' and 39°03' north latitude and 38°45' and 39°08' east longitude (T.C. Malatya Governorate, 2025a).

According to the 2024 Address-Based Population Registration System results, Malatya's total population was 750 491 as of December 31, 2024. The largest population resides in Yeşilyurt District with 298 839 people, followed by Battalgazi District with 276 319 people. Furthermore, 376 487 of the population are male and 374 004 are female (Malatya Governorate, 2025b).

Malatya has significant potential in terms of the livestock sector. Due to the presence of extensive pastures suitable for livestock farming in the region (Table 1), small and large livestock farming are prominent. Livestock farming, which is a source of livelihood or additional income for a significant portion of the population in Malatya, is also important in terms of supplying inputs to many branches of the industrial sector. The Eastern Anatolia Region, where livestock farming is intensively practiced, accounts for 24.4% of large livestock and 34.3% of small livestock. This is due to the presence of extensive pastures in the region (Malatya Provincial Directorate of Agriculture, 2019).

Table 1: *Land Structure and Distribution in Malatya Province (Malatya Governorate 2021, 2024)*

Land Use	2021 (Ha)	%	2024(Ha)	%
Agricultural Land	425.450	34	421.993	34
Pasture	580.423	47	279.235	23
Forest and Scrubland	149.128	12	234.745	18
Other	86.199	7	315.075	25
TOTAL	1.241.200	100	1.251.048	100

Current Situation

In 2021, there are 1.5 billion cattle, 1.3 billion sheep, 975 million pigs, 25.9 billion chickens, and 102 million beehives in the world. Asia is the continent with the largest livestock population in the world. In 2021, 31% of the world's cattle, 98% of buffalo, 62% of poultry, 44% of sheep, and 51% of goats were located in Asia. The Asian continent ranks first, accounting for 44% of the world's total number of beehives, largely due to the influence of India and China (FAOSTAT, 2023; Presidency of the Republic of Turkey, Strategy and Budget Presidency (SBB), 2023).

Global total meat production reached 357.4 million tons in 2021. Poultry meat accounted for the largest share of total meat production at approximately 38.6%, followed by pork at 33.7%, beef at 21.5%, and lamb at 4.6%. In total red meat production, pork accounted for the largest share at 56.4%, followed by beef at 36% and lamb at 7.7%. In 2021, 42.3% of total meat production worldwide took place in Asia, 18.2% in Europe, and 15.2% in North America, while 44.7% of total milk and dairy production took place in Asia, 25.4% in Europe, and 12.2% in North America. Global milk production increased by 27.5% in 2021 compared to 2010, reaching 918 million tons. Despite the difficult conditions during the Covid-19 pandemic, the dairy industry exceeded 900 million tons in milk production for the first time. The increase in milk production is mainly due to the rise in production in regions with milk shortages, particularly in Asia, along with local demand for milk. However, the significant increase in buffalo milk production during the 2010-2021

period has contributed significantly to the increase in total milk production (FAOSTAT, 2023; SBB, 2023).

Table 2: Number of Cattle in Turkey (TUİK, 2025b)

Years	Cattle-Culture (head, %)		Cattle- Cross bred (head, %)		Cattle- Domestic (head, %)		Buffaloes (head, %)		Total
1991	1 253 865	10.16	4 033 375	32.69	6 685 683	54.18	366 150	2.97	12 339 073
1995	1 702 000	14.13	4 776 000	39.65	5 311 000	44.10	255 000	2.12	12 044 000
2000	1 806 000	16.56	4 738 000	43.44	4 217 000	38.66	146 000	1.34	10 907 000
2005	2 354 957	22.15	4 537 998	42.68	3 633 485	34.18	104 965	0.99	10 631 405
2010	4 197 890	36.65	4 707 188	41.09	2 464 722	21.52	84 726	0.74	11 454 526
2015	6 385 343	45.20	7 033 803	40.59	1 874 925	13.27	133 766	0.95	15 427 837
2018	8 419 204	48.89	7 030 297	40.82	1 593 005	9.25	178 397	1.04	17 220 903
2019	8 559 855	47.89	7 554 625	42.27	1 573 659	8.81	184 192	1.03	17 872 331
2020	8 838 498	48.68	7 594 127	41.82	1 530 274	8.43	192 489	1.06	18 157 971
2021	8824784	48.93	7 641 100	42.37	1 384 659	7.68	185 574	1.03	18 036 117
2022	8295825	48.73	7324866	43.03	1231265	7.23	171 835	1.01	17 023 791
2023	8070159	48.67	7303667	44.04	1047430	6.32	161 749	0.98	16 583 005
2024	8213136	48.35	7669922	45.15	941150	5.54	162 052	0.95	16 986 259

In 2021, the global average per animal was 218 kg of beef, 151 kg of buffalo meat, 16 kg of sheep meat, 13 kg of goat meat, 2 692 kg of cow's milk, 1 951 kg of buffalo milk, 41 kg of sheep milk, and 94 kg of goat milk (SBB, 2023).

The number of cattle in our country between 1991 and 2024 is given in Table 2. In Turkey, the number of cattle, which was 12 339 073 in 1991, increased by 37.65% (4 647 186 head) to 16 986 259 head in 2024. Alongside this numerical increase in the number of cattle, there has also been a change in the structural composition. In 1991, 10.16% of the cattle population was purebred, 32.69% was crossbred, 54.18% was local, and 2.97% was buffalo, while in 2024, this structure has completely changed. In 2024, 48.35% of the cattle population was purebred, 45.15% was crossbred, 5.54% was native, and 0.95% was buffalo.

According to 2024 data, cattle in Malatya account for 0.0107% of Turkey's total cattle population (Table 2-3). Compared to 2005, the province saw an increase of 80 554 heads (79.5%) in its total cattle population in 2024. In 2024, 53.52% of Malatya's cattle population was purebred, 44.51% was crossbred, 1.97% was native, and 0.004% was buffalo.

Table 3: *Number of Cattle in Malatya (TUİK, 2025b)*

Years	Cattle-Culture (head, %)		Cattle- Cross bred (head, %)		Cattle- Domestic (head, %)		Buffaloes (head, %)		Total
2005	22 049	21.75	54 477	53.73	24 858	24.52	-	-	101 384
2010	19 998	19.77	65 691	64.95	15 449	15.28	-	-	101 138
2015	47 207	36.21	71 152	54.58	12 012	9.21	-	-	130 371
2016	53 932	39.61	73 772	54.18	8 445	6.20	-	-	136 149
2017	75 994	44.19	85 248	49.57	10 711	6.23	9	0.005	171 962
2018	78 236	44.88	86 562	49.66	9 510	5.46	13	0.007	174 321
2019	85 277	47.21	87 621	48.50	7 738	4.28	13	0.007	180 649
2020	83 366	47.17	86 178	48.76	7 164	4.05	21	0.012	176 729
2021	84 244	48.14	72 765	41.58	6 638	3.79	16	0.009	174 986
2022	82 383	47.40	86 547	49.79	4 880	2.81	10	0.006	173 820
2023	91 874	51.58	81 916	45.99	4 331	2.43	9	0.005	178 130
2024	97 367	53.52	80 975	44.51	3 590	1.97	7	0.004	181 938

The total number of milked animals in our country (Table 4) was 6 660 086 as of 2019, and the total amount of milk produced was 20 861 716 kg. The average milk yield per animal is 1 395.49 kg. of the milked animals, 48.78% are purebred cattle, 41.22% are crossbred cattle, 8.81% are native cattle, and 1.19% are buffalo. In 1991, the number of milked animals was 6,290,079, and the total milk yield was 8,777,760 kg. Again, 10.35% of the milked animals were purebred cattle, 33.20% were crossbred cattle, 53.76% were native cattle, and 2.72% were buffalo. From 1991 to 2019, there was an increase of 370 007 in the number of milked animals, while the increase in milk quantity was 12 083 948 kg, representing an increase of approximately 137.7%. This increase can be attributed to the composition of the number of milked animals. In 1991, the vast majority of milked animals were local breeds with low average milk yield per animal (744 kg), while in 2019, this situation consisted of breeds with high average milk yield per animal (3 861 kg for purebreds and 2 722 kg for crossbreds). Furthermore, this increase can also be attributed to the improvement in record keeping in animal husbandry over the years and the effects of breeding efforts related to the average milk yield of all milked animals.

As of 2019, Malatya province accounts for 1% of the total number of milked animals in Turkey in terms of the total number of milked animals (Table 4-5). Comparing 2005 and 2019, there was a 32.06% increase (16 244 heads) in the number of milked animals and an approximate 54.54% increase (74 488 kg) in the total amount of milk produced (Table 5). As can be seen from Tables 4 and 5, purebred and crossbred cattle account for a significant proportion of milked animals. This explains the increase between 2005 and 2019. The average milk yield per milked animal was found to be similar to the Turkish average for all breeds.

Table 4. *Milk Production Per Head of Cattle in Turkey by Year (TUİK, 2025b)*

Years	Cattle-Culture			Cattle- Cross bred			Cattle- Domestic			Buffaloes		
	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)
1991	650739	1913438	2940	2087014	4188398	2007	3381244	2514576	744	171082	161348	943.10
1995	870248	2581711	2967	2392621	4751023	1986	2622717	1942578	741	122372	114534	935.94
2000	904849	2639113	2917	2335119	4591861	1966	2039601	1501067	736	69602	67330	967.35
2005	925618	3596017	3885	1717309	4646857	2706	1355170	1783328	1316	38205	38058	996.15
2010	1626412	6309065	3879	1787012	4861835	2721	948417	1247644	1316	35362	35487	1003.53
2015	2500880	9672573	3868	2314061	6315366	2729	720833	945581	1312	62999	62761	996.21
2016	2542163	9825300	3865	2235501	6101826	2730	654051	859137	1314	63329	63085	996.14
2017	2940907	11355933	3 861	2426764	6620540	2728	601377	785846	1307	69497	69401	998.61
2018	3185959	12301080	3861	2554947	6957715	2723	597001	778082	1303	75882	75742	998.15
2019	3249002	12544507	3861	2745243	7473837	2 722	586508	764031	1303	79333	79341	1000.09

According to the latest statistics (for 2019), the average milk production per animal in our country was 3.86 tons for purebred cattle, 2.72 tons for crossbred cattle, 1.30 tons for native cattle, and 1 ton for buffalo. For Malatya, these values are 3.83 tons for purebred cattle, 2.75 tons for crossbred cattle, 1.37 tons for native cattle, and 1 ton for buffalo (Table 4-5). While the average milk yield per cow in our country is generally above the world average, it is quite low compared to some countries with developed livestock industries (such as some European countries or the USA) (SBB, 2023).

Table 5. *Milk Production Per Head of Cattle in Malatya by Year (TUİK, 2025b)*

Years	Cattle-Culture			Cattle- Cross bred			Cattle- Domestic			Buffaloes		
	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)
2005	10640	40762.33	3831.04	30803	83161.69	2699.79	9213	12667.29	1374,93	0	0	0
2010	11054	42347.79	3830.99	35422	97622.59	2755.98	6242	8582.97	1375,03	0	0	0
2015	18206	69745.96	3830.93	31445	86662.86	2756.01	4926	6773.53	1375,05	0	0	0
2016	18820	72100.99	3831.08	30691	84309.95	2747.05	3444	4735.21	1374,91	0	0	0
2017	27363	104829.14	3831.05	33678	92816.81	2756	4802	6602.18	1374,88	3	3.36	1120.67
2018	28611	109606.90	3830.93	34305	94544.33	2755.99	4316	5934.67	1375,04	5	5.04	1008.60
2019	29368	112510.60	3831.06	34006	93721.58	2756.03	3526	4847.74	1374,85	5	5.04	1008.60

In 2024, a total of 32 828 493 animals were slaughtered in our country, yielding 2 105 895 tons of red meat. In the previous year (2023), 38 072 586 animals were slaughtered, yielding 2 384 047 tons of red meat (Table 6). Red meat production in 2023 decreased by 11.7% in 2024. The distribution of this decrease in red meat production by type is as follows: beef production decreased by 11.2%, mutton production decreased by 10.5%, goat meat production decreased by 22.8%, and buffalo meat production decreased by 10.4%. Of the 2,105,895 tons of red meat produced in our country in 2024, 70.42% was beef, 0.65% was buffalo, 24.20% was sheep, and 4.73% was goat meat. As of 2024, the average meat production per animal is 292.06 kg for cattle, 229.81 kg for buffalo, 22.57 kg for sheep, and 19.45 kg for goats (Table 7).

Table 6: *Number of Cattle Slaughtered by Type and Year in Turkey, Amount of Red Meat Produced (Tons), and Average Carcass Yield per Animal (kg) (TUİK, 2025b)*

Years	Buffalo and Malak				Cattle or Calf				Total (Ton)
	Head	Ton	Kg	%	Head	Ton	Kg	%	
2001	36072	6486	179.81	0.83	2832912	493763	174.30	63.03	500249
2005	25060	4629	184.72	0.63	2558207	491560	192.15	66.68	496189
2010	19126	3785	197.90	0.43	2932054	647067	220.69	73.55	650852
2015	25713	5300	206.12	0.45	3706346	862098	232.60	72.63	867398
2016	27663	5470	197.74	0.42	3993893	956180	239.41	73.35	961650
2017	29476	5868	199.08	0.41	4334034	1093841	252.38	75.94	258252
2018	32389	6515	201.15	0.39	4844711	1281234	264.46	77.10	1287749
2019	35695	7150	200.31	0.41	4856517	1330169	273.89	76.42	1337319
2020	40929	8424	205.82	0.47	4812902	1341446	278.72	75.11	1349870
2021	51925	10831	208.59	0.55	5134441	1460719	284.49	74.83	1471550
2022	62285	13586	218.13	0.62	5480489	1572747	286.97	71.76	1586333
2023	69597	15386	221.07	0.65	5811698	1670606	287.46	70.07	1685992
2024	59965	13781	229.82	0.65	5077812	1483042	292.06	70.42	1496823

Red meat production data for Malatya province is quite limited. According to data from the Malatya Provincial Directorate of Agriculture and Forestry, in 2020, the number of cattle slaughtered was 24094, the carcass weight obtained was 7 367 457 kg, In 2021, the number of cattle slaughtered was 22 729, and the carcass yield was 7 111 119 kg. In 2023, the number of cattle slaughtered was 11830, and the carcass yield was 3,759,166 kg. Small livestock data shows that in 2020, 12 801 heads and 293 670 kg of carcass were obtained, in 2021, 4 758 heads and 145 194 kg of carcass were obtained, and in 2023, 10 869 heads and 266 200 kg were obtained. The average carcass yield per animal in 2023 is 317.77 kg for large livestock and 24.49 kg for small livestock (Malatya Governorate 2021, 2024).

Table 7: *Number of Small Ruminants Slaughtered by Type and Year in Turkey, Amount of Red Meat Produced (Tons), and Average Carcass Yield per Animal (Kg) (TUİK, 2025b)*

Years	Goat				Sheep				Total
	Head	Ton	Kg	%	Head	Ton	Kg	%	
2001	3135214	57537	18.35	7.35	12450811	225555	18.11	28.79	283092
2005	2801617	50492	18.02	6.85	10731398	190539	17.75	25.85	241031
2010	2244760	42846	19.08	4.87	9691041	186121	19.20	21.15	228967
2015	4097340	69757	17.02	5.88	12808697	249863	19.50	21.05	319620
2016	4346611	75322	17.32	5.78	13277503	266675	20.08	20.46	341997
2017	4346713	77794	17.89	5.40	13244903	262825	19.84	18.25	340619
2018	4392427	82839	18.85	4.98	14133170	291179	20.60	17.52	374018
2019	4513264	87126	19.30	5.01	14546576	316170	21.73	18.16	403296
2020	4692010	90443	19.27	5.06	15801021	345639	21.87	19.35	436082
2021	4907371	94555	19.26	4.84	17125163	385933	22.53	19.77	480488
2022	6112179	115938	18.96	5.29	21563828	489354	22.69	22.33	605292
2023	6753478	128989	19.09	5.41	25437813	569066	22.37	23.87	698055
2024	5117030	99532	19.45	4.73	22573686	509539	22.57	24.20	609071

Small ruminant farming is one of the most important branches of animal husbandry that our country's farmers have engaged in throughout history. It plays a significant role in our country's economy in terms of meat, milk, wool, and leather production. It has been an important source of livelihood, especially for the people living in the Eastern Anatolia and Southeastern Anatolia regions. The number of small ruminants in our country is given in Table 8. According to 2024 data, there are 54902668 small ruminants in Turkey. Sheep constitute 80.29% (44 080 584 head) of the small ruminant population, while goats constitute 19.71% (10 822 084 head). Table 7 shows that there has been an increase of approximately 72.5% (23 080 879 heads) in the number of small ruminants between 2005 and 2024. Although there has been an increase in the number of small ruminants over the last 20 years, there has been a decline compared to previous years. In 1980, there were 64.8 million head of livestock in our country, and compared to the number of small ruminants in 2024, there has been a decrease of approximately 15.3% (9 898 332 head) (TİGEM, 2023). Furthermore, Turkey's population in 1980 was 44 736 957 (DPT, 1982), while its population in 2024 is 85 664 944 (TUİK, 2025a). While there were approximately 1.5 small ruminants per person in the country in 1980, this figure has fallen to 0.64 in 2024. As mentioned above, small ruminant breeding, which has an important place in our history, must be given the importance it deserves and should be seen as the starting point for the country's livestock industry.

Table 8: *Turkey Small Ruminant Numbers (TUİK, 2025b)*

Years	Sheep-Merinos		Sheep-domestic		Goat- Mohair		Goat Hair and Others		Total (Head)
	Head	%	Head	%	Head	%	Head	%	
2005	752353	2.36	24551972	77.15	232966	0.73	6284498	19.74	31821789
2010	1086392	3.70	22003299	74.88	152606	0.52	6140627	20.89	29382924
2015	2205576	5.26	29302358	69.89	205828	0.49	10210338	24.35	41924100
2016	2151264	5.21	28832669	69.76	207765	0.50	10137534	24.52	41329232
2017	2420228	5.46	31257408	70.54	215645	0.49	10419027	23.51	44312308
2018	2681679	5.81	32513293	70.50	223874	0.49	10698553	23.19	46117399
2019	3076583	6.35	34199467	70.54	241055	0.50	10964374	22.61	48481479
2020	3547033	6.55	38579748	71.30	287020	0.53	11698825	21.61	54112626
2021	3994791	6.95	41182899	71.60	289557	0.50	12051957	20.95	57519204
2022	3958934	7.04	40728954	72.39	257654	0.46	11320208	20.11	56265750
2023	3851835	7.36	38208635	72.97	210184	0.40	10092756	19.27	52363410
2024	4208732	7.67	39871852	72.62	202243	0.37	10619841	19.34	54902668

As of 2024, Malatya province accounts for 0.69% of the total number of small ruminants in Turkey (Table 7-8). Within the total number of small ruminants, sheep account for 86.12% and goats account for 13.88%. Compared to 2005, there has been an increase of 152 590 heads (67.25%) in the number of small ruminants in 2024 (Table 9).

Table 9: *Malatya Turkey Small Ruminant Numbers (TUİK, 2025b)*

Years	Sheep-Merinos		Sheep-domestic		Goat- Angora		Goat Hair and Others		Total (Head)
	Head	%	Head	%		Head	%	Head	
2005	0	-	189219	83.40	0	-	37653	16.60	226872
2010	0	-	200564	83.93	0	-	38417	16.07	238981
2015	94	0.03	236334	78.20	0	-	65771	21.77	302199
2016	122	0.04	225522	78.86	0	-	60347	21.10	285991
2017	146	0.04	269172	79.18	0	-	70669	20.78	339987
2018	311	0.09	268911	79.46	0	-	69211	20.45	338433
2019	334	0.09	285780	79.83	0	-	71904	20.08	358018
2020	518	0.14	294517	81.91	0	-	64564	17.95	359599
2021	761	0.21	301843	82.11	0	-	65002	17.68	367606
2022	492	0.13	307245	83.21	0	-	61525	16.66	369262
2023	152	0.04	326305	85.24	0	-	56346	14.72	382803
2024	161	0.04	326657	86.08	0	-	52644	13.88	379462

Looking at the years (1991-2024), the change in the number of milked sheep and goats in our country is as follows: a 1.95% increase in hair goats (102 950 heads), an 84.6% decrease in angora goats (509354 heads), a 19.28% decrease in native sheep (4 383 116 heads), a 203.5% increase in Merino sheep (997 856 heads), and a 13.03% decrease in total small ruminants (3 791 664 heads) (Table 9). In terms of average milk yield per animal, there was a 74.75% increase in hair goats (43.63 kg), a 75.7% increase in angora goats

(15.90 kg), a 61.7% increase in native sheep (30.13 kg), and a 40.55% increase in merino sheep (13.97 kg). As with cattle, this increase can be attributed to improvements in record-keeping in animal husbandry over the years and the effects of breeding efforts related to the average milk yield of all individuals among milked animals.

Table 10. Number of small ruminants milked and milk production in Turkey (TUİK, 2025b)

Years	Sheep- Domestic			Sheep-Merinos			Goat- Ordinary			Goat- Angora		
	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)
1991	22731840	1110534	48.85	490405	16909	34.47	5275399	322084	61.05	602091	12655	21.01
1995	18801878	918495	48.85	460615	16005	34.74	4544493	269670	59.34	363091	7537	20.75
2000	15489474	759875	49.05	430685	14504	33.67	3604719	216328	60.01	187988	3883	20.65
2005	9837155	774344	78.71	328936	15533	47.22	2331556	250246	107.33	95437	3513	36.80
2010	10070029	792122	78.66	513579	24710	48.11	2516200	270476	107.49	66339	2335	35.19
2015	14348611	1129237	78.70	1014316	47990	47.31	4483672	477824	106.56	94822	3350	35.32
2016	14160816	1113469	78.63	988598	46943	47.48	4466406	476234	106.62	88699	3167	35.70
2017	16330147	1288041	78.87	1173267	56738	48.35	4877554	520197	106.65	86027	3198	37.17
2018	17497602	1382026	78.98	1321682	64245	48.60	5234796	558418	106.67	92370	3408	36.89
2019	18348724	1449351	78.98	1488261	72105	48.44	5378349	573786	106.68	92737	3423	36.91

As of 2019, Malatya province accounts for 0.83% of the total number of small ruminants milked in Turkey (Table 9-10). The proportion of domestic sheep milked is 82.52% (173 391 head), while the proportion of hair goats milked is 17.38% (36 508 head). As can be seen from the table, native sheep and hair goat breeding is widespread in Malatya Province (Table 11).

Table 11. Number of small ruminants milked and milk production in Malatya (TUİK, 2025b)

Years	Sheep- Domestic			Sheep-Merinos			Goat- Ordinary			Goat- Angora		
	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg)	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg	Number of animals milked (head)	Milk (tons)	Average milk yield per animal (kg
2005	119330	9307.70	77.99				17416	1619.69	93			
2010	127504	9945.33	78.00				19351	1799.59	93			
2011	137870	10753.84	77.99	143	7.12	49.83	21213	1972.83	93	95	2.47	26
2012	138015	10765.17	78.00	99	4.94	49.90	26472	2461.93	93			
2013	134209	10468.33	78.00	90	4.51	50.14	29022	2699	93			
2014	122989	9593.13	77.99	38	1.90	50.00	28931	2690.61	93			
2015	147439	11500.25	78.00	29	1.42	49.14	33378	3104.12	93			
2016	149345	11648.89	77.99	43	2.13	49.72	32307	3004.57	93			
2017	167173	13039.53	78.00	38	1.90	50.00	37654	3501.80	93			
2018	166974	13023.96	77.99	182	9.12	50.11	37352	3473.69	93			
2019	173391	13524.51	78.00	226	11.30	50.02	36508	3395.25	93			

The total milk produced from small ruminants milked in Turkey in 2019 was 573 786 tons from hair goats, 3 423 tons from angora goats, 1 449 351 tons from native sheep, 72 105 tons from merino sheep, and 2 098 665 tons in total (Table 10). The total milk produced in Malatya province accounts for 0.807% of the milk produced in Turkey (Tables 10-11).

In particular, the very low average milk yield per animal in both Turkey and Malatya can be attributed to the low milk yield of milked animals, breeding methods, the use of local genotypes, and the failure to maintain adequate records.

Problems and Proposed Solutions

Turkey's large and small ruminant livestock composition is similar to that of Malatya province. While the vast majority of large livestock are crossbred (93.5% crossbred in Turkey, 98% crossbred in Malatya), the situation is reversed for small livestock, where native breeds predominate (92.33% native in Turkey, 100% native in Malatya). The problems related to livestock and animal production in Malatya Province are essentially similar to those in the country as a whole. Problems related to large and small livestock and proposed solutions are given below.

1. National Registration; The "Animal Registration System" is a centralized system in our country that performs identification, registration, monitoring,

and tracking of animals from birth to death in an electronic environment. It records where animals are born, their breed, age, sex, farm information, movements (transfer from one farm to another, etc.), vaccinations, and events such as birth, death, and slaughter. Thanks to this system, both animal owners and the government can manage information such as animal stock, movement, production, and health status in a secure and organized manner. Again, thanks to this system, livestock-related policies can be easily created and managed. However, it has been observed that there are still shortcomings and problems in our National Registration system. These must be overcome. But most importantly, in order to increase the effectiveness of solutions, it is necessary to urgently reveal the clear data on the country's livestock. This requires a new animal census (breed, age, gender, farm information, movements (transfer from one farm to another, etc.), vaccinations, birth–death–slaughter, etc.) to be carried out in all its aspects.

2. Sustainable Livestock Farming: Reducing input costs (providing fertilizers, fuel, seeds, medicines, feed, electricity, etc. to producers at affordable prices) and increasing revenues (expanding the range of products produced and ensuring that products are sold at their true value).

3. Increasing the Age of Operations; Reducing the age of livestock farmers, encouraging young people (especially women) to engage in livestock farming, ensuring that at least one member of livestock farming families is considered public personnel and receives a salary, providing livestock farmers with benefits that improve their quality of life, such as social, educational, health, and sports opportunities, ensuring the continuity of existing businesses to increase the age of the business,

4. Trained Personnel: Improving the level of knowledge of individuals responsible for each stage of breeding and making them experts in their field.

5. Effectiveness of livestock support; Providing agricultural support in an amount not less than 1% of the GNP, as stated in the Agriculture Law, increasing the share of livestock in agricultural support, determining the support to be provided in advance and providing it on time, preventing the use of existing support for purposes other than those intended,

6. Yield records; Evaluating both businesses and the country's livestock sector, presenting the current situation, and developing policies that will generate problems and proposed solutions, ensuring that records are kept of all items (fuel, seeds, medicines, feed, electricity, etc.) of livestock businesses and all yields obtained from animals (live weight, amount of feed given, milk, fertility, etc.).

7. Care, feeding, and housing procedures: The ability of animals to express their genetic potential depends on environmental conditions (such as care, feeding, and housing). This issue is one of the most significant problems in our country's livestock industry. For animals to fully express their genetic potential, those involved in livestock farming must have sufficient knowledge and experience in this area. Taking into account the factors affecting animal housing (such as breed genetic characteristics, season, geography), it is necessary to create housing models that are sufficiently comfortable and suitable for the conditions of the region, and to prepare rations (roughage-concentrate) that will fully meet the needs of the animals (such as living allowance, yield allowance, pregnancy allowance) and providing them to the animals at the appropriate time. Furthermore, producers must fully carry out the care and management tasks during the breeding period (dry period, pre-breeding period, breeding period, birth, post-birth, etc.).

8. Feed Sources: In livestock farming, feed accounts for approximately 70% of expenses in the years following the establishment of the operation. This directly highlights the importance of feed sources and nutrition in livestock farming. The needs of the country's livestock sector must be determined, and feed crop planting and production planning must be carried out accordingly. Special production areas should be established for the production of high-quality roughage. Pastures are the most important natural source of roughage, which can be provided in large quantities and at low cost, but our pasture areas have been degraded as a result of undisciplined and careless use. The sustainable use of pastures must be ensured. Livestock farming on pastures is the cheapest method in terms of cost. In particular, to address the roughage deficit, meadows should be protected, pastures should be improved, and the grazing capacity of these areas should not be exceeded. The state should develop incentive policies for forage crop production by promoting irrigation (Aysöndü and Koçyiğit 2016). Exorbitant increases in roughage prices should be prevented. Increases in concentrate feed prices linked to imports and exchange rates should be subsidized at the producer level. In addition, producers/farmers should be informed about all technical and technological applications for the storage and preservation of roughage, and some applications should be made mandatory (closed roughage storage, silage pits, use of mycotoxins). Sufficient quantities should be produced in feed sources.

9. Animal movements: Maximum border security must be ensured to protect our animals from disease, prevent the spread of disease, and prevent disease from causing losses among animals. Maximum care must be taken at border controls, and the illegal entry of animals into our country must be strictly prevented. In addition, mobile slaughterhouses and illegal slaughtering

in border provinces must be prevented. If there is a risk of an epidemic or zoonotic disease in any region, animal movement must be strictly prevented. To this end, animal movements must be strictly controlled.

10. Animal production and animal products: The state must urgently take measures to protect the products produced by producers (primarily meat and milk). Unregistered and underground production must be prevented. Organizations should be established and managed for producers in areas such as product preservation, transportation, processing, branding, and marketing, or management mechanisms should be created. To this end, regional shared machine parks should be established with the necessary tools and equipment to ensure that producers benefit maximally from technology.

11. Reducing losses due to animal diseases; Insufficient veterinary health services in our region and neighboring countries, inadequate border security, and deficiencies in the control of animal markets and animal movements have led to the spread of epidemic and contagious animal diseases through animal imports. Unfortunately, the damage caused to producers and the national economy due to these diseases has reached enormous proportions today. In particular, measures must be taken to prevent the high mortality rates among young animals (calves 5-60%, lambs and kids 4-21%), starting with assistance during birth and including care, feeding, housing, and vaccinations, with the aim of reducing mortality to a minimum. Furthermore, the number of disease-free operations, particularly with regard to mastitis, foot-and-mouth disease, tuberculosis, and brucellosis, should be increased. Preventive veterinary medicine should be the guiding principle. Biosecurity measures, such as the administration of vaccines against epidemic diseases, the proper transportation of animals, and the disposal of sick/dead animals, should be implemented without compromise. Special sanctions should be introduced to enforce biosecurity measures (such as penalties or withdrawal of support for non-compliance). The necessary work should be completed to produce domestic medicines and vaccines, and dependence on foreign sources should be eliminated or reduced.

12. Supporting sheep and goat farming; Since we can meet our meat deficit most quickly through small ruminant farming, small ruminant farming should be supported. With the development of pasture farming, sheep and goat farming, which makes the best use of pastures, should be increased. Misconceptions about sheep and goat meat consumption should be eliminated. The social security and social lives of shepherds should be improved. The Social Security Institution can make regulations to evaluate these workers based on the number of hours they should work per year, rather than per week. The use of different breeding techniques (artificial insemination and embryo transfer) in small ruminant farming should be promoted. Projects aimed at obtaining

three twin/triplet lambs in two years should be implemented in conscious and economical enterprises.

13. Organizational mechanism; Livestock organizations enable those engaged in livestock farming to join forces and demonstrate their effectiveness or power (by collectively purchasing all the producer's needs, such as fuel, feed, fertilizer, and seeds, at below market prices to reduce input costs, providing even the smallest producer with access to the latest technology through a shared machine park, increasing efficiency through on-site training and supervision at members' businesses, creating added value by processing producers' products into finished goods, paving the way for branding, etc.). Their management and activities should be effectively monitored by the state, and all necessary measures should be taken to ensure that the organizations do not deviate from their purpose. Furthermore, a comprehensive, effective, mobile, and centrally coordinated General Directorate of Veterinary Affairs must be established without fail and should play an active role in public-private sector cooperation.

14. The unreliability of agricultural statistics; The last genuine agricultural census in our country was conducted in 2001. Currently, there are significant inconsistencies in animal and plant production figures. The accurate planning of animal production is linked to the accuracy of plant and animal production figures.

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Chapter 3

Effects of Dietary Crude Glycerin on Internal Organ Development, Muscle Composition, and Meat Quality in Broilers[1]

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1 This manuscript is derived from the first author's doctoral dissertation entitled "*The Effects of Crude Glycerin Used in Broiler Mixed Feeds on Performance, Small Intestine Histopathology, and Footpad Lesions*". Advisor: Prof. Dr. Ergün DEMİR

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Introduction

As the demand for animal-based foods continues to rise globally, the search for affordable, sustainable, and environmentally conscious feed ingredients in poultry diets has gained importance. Cereal grains constitute the predominant energy source in broiler diets; however, the rising costs of conventional energy ingredients have prompted the exploration of alternative sources (Garcia et al., 2018; Bölükbaş & Kaya, 2022). In this context, crude glycerin (CG), which is obtained as a by-product during biodiesel production, has attracted considerable attention due to its high energy value and digestibility (Topal & Ozdogan, 2013; Silva et al., 2019).

Crude glycerin is a complex mixture composed mainly of glycerol, water, methanol, soaps, and residual fats. Glycerol can be readily absorbed and directly enter carbohydrate and lipid metabolic pathways, allowing partial replacement of corn or fats as an energy source (Światkiewicz & Koreleski, 2009; Topal & Ozdogan, 2013). However, its compositional characteristics are largely determined by the type of feedstock used (e.g., vegetable or animal oils) and the purification method applied (de Souza et al., 2020; Bölükbaş & Kaya, 2022). Therefore, a detailed understanding of its chemical composition and physiological implications is essential to ensure its safe and efficient utilization in animal nutrition (Alvarenga, 2012; Sehu et al., 2013).

Evidence from earlier studies suggests that moderate dietary inclusion of CG has no detrimental influence on broiler growth performance or carcass characteristics (Topal & Ozdogan, 2013; Legawa et al., 2018; Silva et al., 2019; de Souza et al., 2020). Beyond serving as an energy source, glycerol may enhance muscle water-holding capacity and osmotic balance, contributing to improved meat quality (Dransfield & Sosnicki, 1999; Brossi et al., 2009). However, excessive use of low-purity CG may exert negative physiological effects due to residual methanol or soap content (Garcia et al., 2018; Urgnani et al., 2014).

Most researcher to date has focused on the influence of CG on growth performance and blood parameters, while information regarding its effects on internal organ development, muscle chemical composition, pH, and meat color characteristics remains limited (Silva et al., 2019; Sopian et al., 2020; de Souza et al., 2020). A better understanding of these parameters is crucial to assess CG not only as an energy substitute but also as a potential modulator of muscle physiology and sensory meat properties (Işık, 2008; Şekeroğlu & Diktaş, 2012).

Therefore, the objective of this experiment was to investigate the effects of dietary inclusion of crude glycerin at different levels on internal organ weights,

physicochemical characteristics (pH and color), and muscle composition of broiler meat. The findings are expected to contribute to defining the safe inclusion levels of CG as an alternative energy source and to support the development of sustainable feeding strategies in broiler production.

Materials and methods

The study was carried out at the Broiler Research Unit, Balıkesir University, using 350 Cobb 500 broilers of both sexes. For the first four days, all birds received a pre-starter diet based on soybean meal-corn, formulated according to NRC (1994). On day five, the chicks were randomly allocated to seven dietary treatments, each group including 10 birds and replicated five times, under a completely randomized design with an equal male-to-female distribution within each replicate (Table 1). Throughout the experimental period, the birds had *ad libitum* access to water and their respective diets. Ethical approval for the experiment was granted by the Balıkesir University Animal Ethics Committee (HADYEK) under protocol number 2023/1-3.

Table 1. *Diet type of the groups in the study*

Group I	Days 1-42 Control (Corn–soybean meal–based basal diet)
Group II	Days 5-21 5% CG + days 22-42 0% CG
Group III	Days 5-21 0% CG +days 22-42 5% CG
Group IV	Days 5-42 5% CG
Group V	Days 5-21 10% CG + days 22-42 0% CG
Group VI	Days 5-21 0% CG + days 22-42 10% CG
Group VII	Days 5-42 10% CG

The crude glycerin used in this study was analyzed following the official methods of the Association of Official Analytical Chemists (AOAC, 1997). Its composition was calculated based on the relationship: [Glycerol+Moisture+Ash+Matter Organic Non-Glycerol (MONG)]=100. The analyzed sample contained glycerol 87.48%, moisture 6.82%, ash 4.00%, and MONG 1.70%. The CG originated from biodiesel production based on sunflower and canola oils. The methanol concentration in crude glycerin was measured at 0.3% according to the analytical procedure outlined by Dozier et al. (2011). The gross energy (GE) value of the sample was assessed with an adiabatic bomb-calorimeter (IKA C6000 Global Standard, Germany) and found to be 15.83 MJ/kg (approximately 3,784.4 kcal/kg). The apparent metabolizable energy corrected for nitrogen (AMEn) was estimated as 95% of GE, corresponding to 3,595 kcal/kg (Dozier et al., 2008). The chemical composition of CG was further analyzed to contain 0.07% crude protein (CP) and 0.10% ether extract (EE) (AOAC, 1997). Chloride (Cl^-) and Sodium (Na^+) concentrations were determined as 1.99% and 1.29%, respectively, according to AOAC (1997). The pH value of the sample was measured with an Orion Star™ A111 pH meter (Thermo Scientific, USA), and the obtained reading was

4.35. The experimental diets were prepared to maintain consistent protein and energy levels (isonitrogenous and isocaloric) across the four feeding phases—days 1–4, 5–10, 11–21, and 22–42—based on the nutrient recommendations of NRC (1994). The proximate composition analyses of diets, including crude ash, dry matter (DM), CP, and EE were conducted following AOAC (1997). Diets (mash-form) are presented in Table 2.

At day 42 of the experiment, two broilers were randomly selected from each replicate, resulting in a total of 70 birds for various analyses. The selected birds were fasted for 12 hours prior to slaughter and euthanized by decapitation. Following slaughter, internal organs including the liver, gizzard, spleen, heart, proventriculus, pancreas, and intestines were carefully removed and weighed individually. The relative organ weights were calculated as grams per 100 g of live body weight. In addition, the abdominal fat content was determined according to the procedure described by Kubena et al. (1974).

The color and pH characteristics of the breast and drumstick muscles were determined according to the methods described by Legawa et al. (2018) and de Souza et al. (2020). For this purpose, the right side of the breast muscle and the right drumstick of each slaughtered bird were used for the analyses. Muscle pH was measured at 15 minutes and 24 hours postmortem using a pH meter (Mettler Toledo-Seven2Go pH meter), with readings taken from two different points of each muscle. The mean of these two measurements was recorded as the final pH value for each sample. The color of the skinless drumstick and breast muscles was measured using a colorimeter (Konica Minolta Chromometer, Model CR-300). Color parameters L^* (lightness), a^* (redness), and b^* (yellowness) were recorded from two different surface points on each muscle, and their averages were considered as representative color values for breast and drumstick meat. Additionally, the left drumstick and breast muscles from one bird per replicate were collected immediately after slaughter to determine their chemical composition. Samples were stored at −18°C until analysis. Prior to chemical analysis, frozen samples were minced into small pieces and homogenized using a blender. Moisture, CP, EE, and ash contents were determined following AOAC (1997) procedures.

Table 2. *Ingredient and nutrient composition of diets (as-fed basis)*

	Prestarter (Days 1-4).		Starter (Days 5-10)		Grower (Days 11-21)		Finisher (Days 22-42)	
Ingredients %								
Soybean meal, 46% CP	36.60	33.54	34.65	35.73	31.15	32.17	33.19	28.90	30.185	30.20
Corn grain	55.53	58.91	53.00	47.00	59.00	52.49	46.00	61.40	56.99	48.73
Sunflower Seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.87
Corn Oil	2.74	2.24	2.14	2.06	3.35	3.37	3.38	4.00	3.62	3.00
Crude Glycerin	0.00	0.00	5.00	10.00	0.00	5.00	10.00	0.00	5.00	10.00
Razmol	1.45	1.41	1.50	1.68	3.00	3.66	4.30	2.62	1.31	2.50
Dicalcium phosphate	1.63	1.68	1.69	1.69	1.505	1.50	1.49	1.31	1.34	1.31
Sodium chloride	0.38	0.38	0.22	0.06	0.38	0.22	0.06	0.39	0.23	0.05
Limestone	1.06	1.05	1.05	1.04	1.02	1.02	1.02	0.94	0.92	0.92
L-Lysine HCL	0.11	0.21	0.19	0.17	0.12	0.10	0.08	0.02	0.00	0.00
L-Threonine	0.01	0.06	0.06	0.05	0.02	0.01	0.01	0.00	0.00	0.00
DL-Methionine	0.23	0.27	0.27	0.27	0.21	0.21	0.21	0.16	0.16	0.16
Vit.-Min.premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated nutrient composition										
Dry Matter (DM)	90.02	89.96	90.15	90.34	89.99	90.18	90.38	89.99	90.17	90.29
ME (kcal/kg)	3035	3035	3035	3035	3108	3108	3108	3180	3180	3180
EE (%)	4.51	4.06	3.84	3.63	5.19	5.08	4.96	5.85	5.34	5.70
CP (%)	22.02	21.04	21.04	21.04	20.04	20.04	20.04	19.02	19.02	19.02
Ash (%)	6.31	6.19	6.22	6.25	5.89	5.93	5.97	5.50	5.51	5.55
Lysine (%)	1.32	1.32	1.32	1.32	1.19	1.19	1.19	1.05	1.05	1.05
Methionine+Cystine (%)	0.98	0.98	0.98	0.98	0.89	0.89	0.89	0.82	0.82	0.82
Calcium (%)	0.90	0.90	0.90	0.90	0.84	0.84	0.84	0.76	0.76	0.76
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.38	0.38	0.38
Chlorine (%)	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1]Contained per kilogram; vitamin D_3: 5.000 000 IU, vitamin K_3: 3 g, vitamin E: 70. 000 IU, vitamin A:9.000 000 IU, tiamin: 3 g; riboflavin: 0.6 g; niacin: 4.5 g; pyridoxine: 4 g, Zn: 60 g, Co: 2 g, I: 2 g, Mn: 100 g, Fe 60 g, Se: 0.35 g, Cu: 2 g.

Statistical analyses

In the present study, a total of seven treatment groups, each replicated five times, were randomly allocated according to the experimental design. All statistical analyses were carried out using SPSS software (Version 25.0, IBM Corp., Armonk, NY, USA). Data were first tested for normality and

homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. For datasets meeting the assumptions of normality and homogeneity, comparisons among treatments were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. When data were normally distributed but variances were unequal, one-way ANOVA followed by Dunnett's T3 test was applied. Non-parametric data were analyzed using the Kruskal–Wallis-H test. For repeated measures, muscle effects and their interactions with treatment groups were analyzed using the General Linear Model (GLM) procedure. Associations among the parameters were analyzed using Pearson and Spearman correlation coefficients along with linear regression, and statistical significance was considered at $P < 0.05$ within a 95% confidence interval.

Results

At day 42 of the experiment, internal organ weights were determined from a total of 70 broilers (two birds per replicate). The results for organ weights and the relationships between CG intake and internal organ weights are presented in Table 3 and Table 4. The relative heart weights (g/100 g BW) of broilers in group VII were higher compared to all other groups, whereas no differences ($P>0.05$) were observed among the remaining groups (Table 3). A significant positive correlation ($r=0.474$) was found between heart weight and CG intake (Table 4). Regression analysis revealed a 25.2% causal relationship, indicating that higher CG consumption could contribute to increased heart weight (Table 4). No differences ($P>0.05$) were observed in the relative spleen, gizzard, proventriculus, pancreas, liver and abdominal fat weights among treatment groups (Table 3). Furthermore, no significant correlations were detected between CG intake and gizzard, proventriculus, spleen, and abdominal fat weight (Table 4). Dietary treatments had no effect on the relative liver weights of broilers ($P>0.05$). (Table 3). But, a positive correlation ($r=0.262$) was identified between CG intake and liver weight (Table 4). The relative pancreas weights were not affected by dietary treatments ($P>0.05$) (Table 3). However, CG intake showed a moderate positive correlation ($r=0.328$) and a weak regression ($R^2=10.8\%$) with pancreas weight (Table 4). Differences were observed among the experimental groups regarding the relative intestinal weights ($P<0.05$). Group VII had significantly higher small and large intestine weights compared to all other groups (Table 3). Additionally, group VII showed higher intestinal weights than groups I, II, III, IV, and V ($P<0.05$) (Table 3). No differences ($P>0.05$) were found between group VI and other groups. A positive correlation ($r=0.407$) and a regression relationship ($R^2=26.8\%$) were observed between CG intake and intestinal weights, indicating that increased CG consumption may lead to higher intestinal development (Table 4).

At day 42, the chemical composition of drumstick and breast muscles was determined using one bird per replicate (35 birds in total), whereas pH and color parameters were measured in two birds per replicate (70 birds in total). The relationships between CG intake and the chemical composition, color characteristics, and pH of the breast and drumstick muscles are presented in Tables 5-8. No differences ($P>0.05$) were found among the experimental groups for ash content in either breast or drumstick muscles (Table 5). However, a significant difference ($P<0.05$) was observed between the two muscle types, with drumstick muscles exhibiting lower ash content compared to breast muscles. No significant correlations were identified between CG intake and the ash levels of breast or drumstick muscles (Table 6). The DM content of breast muscles in group I was higher ($P<0.05$) than in group III and IV, while no differences were found among the remaining groups ($P>0.05$) (Table 5). In the drumstick muscle, group I also exhibited significantly higher DM levels compared to other groups ($P<0.05$). A difference ($P<0.05$) was observed between muscle types, with drumstick muscles showing lower DM content than breast muscles. No meaningful association was found between CG intake and the dry matter content of either muscle (Table 6). Breast and drumstick muscle CP content tended to be lower in groups I and II compared with other treatments, and differences were significant ($P<0.05$) (Table 5). A difference ($P<0.05$) between muscle types was observed, with drumstick muscles containing less protein than breast muscles (Table 5). CG intake showed strong positive correlations with both breast and drumstick CP levels ($r=0.703–0.723$) and moderate regression relationships ($R^2=0.494–0.523$), indicating that increasing glycerol intake enhanced muscle protein deposition (Table 6). The treatments had no effect ($P>0.05$) on EE levels in either breast or drumstick muscles (Table 5). However, the EE content differed ($P<0.05$) between muscle types, with drumstick muscles containing higher EE levels than breast muscles. Moderate correlations ($r=0.421–0.402$) and weak regression relationships ($R^2=0.178–0.161$) were observed between CG intake and EE content, suggesting that higher CG consumption slightly increased intramuscular fat accumulation (Table 6).

Table 3. *Internal organ weights of experimental groups, g/100 g live weight.*

Groups	Heart Median (min-max)	Intestines Median (min-max)	Liver Median (min-max)	Proventriculus Median (min-max)	Pancreas Median (min-max)	Gizzard Median (min-max)	Spleen Median (min-max)	Abdominal fat±SEM
I	0.53 (0.48-0.66)^{ab}	3.66 (3.31-4.31)^{a}	1.60 (1.50-1.99)	0.41 (0.32-0.46)	0.19 (0.16-0.24)	2.24 (1.87-2.45)	0.08 (0.07-0.08)	1.50±0.09
II	0,.52 (0.40-0.62)^{a}	3.43 (3.22-4.28)^{a}	1.62 (1.54-1.87)	0.38 (0.31-0.45)	0.19 (0.14-0.27)	2.13 (1.82-2.72)	0.08 (0.07-0.09)	1.47±0.07
III	0.58 (0.38-0.74)^{ab}	3.50 (3.09-4.67)^{a}	1.66 (1.58-1.85)	0.38 (0.30-0.47)	0.22 (0.15-0.24)	2.20 (1.81-2.41)	0.08 (0.07-0.08)	1.49±0.06
IV	0,56 (0.46-0.70)^{ab}	3.38 (2.97-5.33)^{a}	1.56 (1.42-2.21)	0.39 (0.30-0.42)	0.21 (0.15-0.30)	1.99 (1.74-2.70)	0.07 (0.07-0.08)	1.57±0.04
V	0.54 (0.45-0.61)^{a}	3.70 (3.26-4.64)^{a}	1.64 (1.26-2.03)	0.33 (0.27-0.70)	0.20 (0.15-0.27)	1.99 (1.60-3.24)	0.07 (0.05-0.09)	1.43±0.09
VI	0.59 (0.52-0.75)^{ab}	4.00 (3.50-4.77)^{ab}	1.74 (1.48-2.06)	0.38 (0.34-0.53)	0.22 (0.15-0.26)	2.14 (2.01-2.31)	0.07 (0.07-0.09)	1.46±0.04
VII	0.65 (0.59-0.97)^{b}	4.67 (3.84-5.25)^{b}	1.81 (1.47-2.34)	0.40 (0.34-0.49)	0.24 (0.17-0.28)	2.19 (1.75-2.33)	0.08 (0.07-0.09)	1.44±0.01
P value	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

SEM: Standard Error of Means, min-max: minimum-maximum

Values are expressed as median (min-max). Different superscripts in the column indicate significant differences ($p<0.00238$, Mann–Whitney U test with Bonferroni correction)

Table 4. *Relationship between crude glycerin consumption and internal organ weights, g/100 g live weight.*

		Heart	Gizzard	Proventriculus	Liver	Pancreas	Spleen	Abdominal Fat	Intestines
Correlation	Correlation coefficient	0.474**	-0.044	0.041	0.291*	0.328**	-0.071	-0.050	0.407**
	Significance (2-tailed)	0,000	0.717	0.737	0.014	0.006	0.561	0.683	0,000
	N	70	70	70	70	70	70	70	70
Linear Regression	Regression R-squared	0.252**	0.002	0.002	0.085*	0.108*	0.005	0.002	0.268**
	Significance (2-tailed)	0.000	0.717	0.737	0.014	0.006	0.561	0.683	0.000
	N	70	70	70	70	70	70	70	70

*** Correlation is significant at the 0.001 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

Spearman correlation was used for heart and intestines. Pearson correlation was used for other organs.

Table 5. *Chemical composition of breast and drumstick muscles (%).*

Groups	Breast Ash (%) ±SEM	Breast DM (%)±SEM	Breast CP (%)±SEM	Breast EE (%)±SEM	Drumstick Ash (%)±SEM	Drumstick DM (%)±SEM	Drumstick CP (%)±SEM	Drumstick EE (%)±SEM
I	1.33±0.10	25.43±0.13a	22.40±0.26b	5.22±0.12	1.14±0.03	24.44±0.26a	22.10±0.38b	6.31±0.08
II	1.41±0.08	25.28±0.19ab	22.45±0.26b	5.30±0.12	1.16±0.07	24.02±0.15b	22.14±0.41b	6.36±0.08
III	1.50±0.14	25.13±0.13b	23.14±0.25a	5.37±0.16	1.16±0.02	23.94±0.16b	22.99±0.28a	6.42±0.15
IV	1.38±0.06	25.03±0.10b	23.18±0.24a	5.41±0.18	1.16±0.04	23.85±0.11b	23.02±0.25a	6.49±0.21
V	1.42±0.07	25.21±0.12ab	23.09±0.25a	5.36±0.14	1.11±0.06	23.96±0.13b	22.95±0.28a	6.37±0.15
VI	1.43±0.18	25.25±0.11ab	23.23±0.14a	5.43±0.17	1.11±0.03	24.03±0.13b	23.17±0.26a	6.50±0.24
VII	1.33±0.01	25.29±0.12ab	23.28±0.14a	5.46±0.24	1.16±0.09	24.11±0.15b	23.24±0.18a	6.51±0.21
P value	>0.05	<0.05	<0.05	>0.05	>0.05	<0.05	<0.05	>0.05
Muscles				<0.05				

SEM: Standard Error of Means, Values in the same column that carry different superscripts indicate significant differences ($P < 0.05$).

Table 6. *Relationship between crude glycerin intake (%) and the chemical composition (%) of drumstick and breast muscles.*

		Breast Ash (%)	Drumstick Ash (%)	Breast DM (%)	Drumstick DM (%)	Breast CP (%)	Drumstick CP (%)	Breast EE (%)	Drumstick EE (%)
Correlation	Correlation coefficient	-0.055	0.006	-0.182	-0.243	0.703**	0.723**	0.421*	0.402*
	Significance (2-tailed)	0.755	0.972	0.295	0.160	0.000	0.000	0.012	0.017
	N	35	35	35	35	35	35	35	35
Linear Regression	Regression R-squared	0.003	0.000	0.033	0.059	0.494**	0.523**	0.178*	0.161*
	Significance (2-tailed)	0.755	0.972	0.295	0.16	0.000	0.000	0.012	0.017
	N	35	35	35	35	35	35	35	35

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 7. *Relationship between glycerin intake (%) and the pH and color traits of breast and drumstick muscles.*

	Correlation			Linear Regression		
	Correlation coefficient	Significance (2-tailed)	N	Regression R-squared	Significance (2-tailed)	N
Breast pH	-0.067	0.583	70	0.001	0.831	70
Breast colour-L	0.181	0.135	70	0.048	0.067	70
Breast colour -a	-0.194	0.107	70	0.038	0.107	70
Breast colour -b	-0.142	0.242	70	0.20	0.242	70
Drumstick pH	-0.376**	0.001	70	0.141**	0.001	70
Drumstick colour-L	-0.138	0.256	70	0.019	0.256	70
Drumstick colour-a	-0.334**	0.005	70	0.139**	0.001	70
Drumstick colour-b	-0.188	0.118	70	0.035	0.118	70

** Correlation is significant at the 0.01 level (2-tailed).

Spearman correlation was used for breast pH, breast color-L and drumstick colour-a. Pearson correlation was used for other organs.

Table 8. *Color and pH values of drumstick and breast muscles.*

Groups	Breast pH Median (min-max)	Breast colour-L Median (min-max)	Breast colour-a±SEM	Breast colour-b±SEM	Drumstick pH±SEM	Drumstick colour-L±SEM	Drumstick colour-a Median (min-max)	Drumstick colour-b±SEM
I	6.22 (6.11-6.35)	51.24 (43.01-54.74)[a]	3.22±0.35	10.23±0.51	6.52±0.03a	52.89±0.60ab	3.21 (2.04-5.28)	11.31±0.32
II	6.25 (6.11-6.28)	48.73 (47.14-51.37)[b]	2.54±0.28	9.77±0.24	6.49±0.04ab	52.96±0.83ab	2.63 (1.14-3.69)	10.45±0.55
III	6.23 (5.98-6.34)	50.31 (43.28-54.54)[a]	2.41±0.25	10.30±0.44	6.45±0.04ab	50.83±0.65b	2.51 (1.27-3.44)	11.49±0.46
IV	6.22 (6.07-6.28)	49.85 (47.23-53.91)[a]	3.28±0.34	10.02±0.36	6.51±0.03ab	53.36±0.91ab	3.15 (1.80-4.82)	11.53±0.36
V	6.26 (6.05-6.31)	51.79 (49.18-54.69)[a]	2.10±0.31	10.16±0.35	6.49±0.04ab	54.76±0.47a	1.80 (1.11-4.30)	11.71±0.41
VI	6.20 (6.12-6.33)	51.93 (47.02-59.15)[a]	2.46±0.21	9.93±0.52	6.42±0.02ab	53.56±0.56ab	2.48 (1.38-3.40)	10.86±0.36
VII	6.21 (6.07-6.39)	51.27 (45.23-55.28)[a]	2.13±0.19	9.40±0.56	6.38±0.03b	51.47±0.94b	2.16 (1.17-3.43)	10.17±0.48
p value	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Muscle				<0.001				

SEM: Standard Error of Means

Values are expressed as median (min-max). Different superscripts in the column indicate significant differences ($p<0.00238$, Mann–Whitney U test with Bonferroni correction)

No differences (P>0.05) were observed in the pH values of breast muscles among the treatment groups (Table 8). However, the pH of drumstick muscles in group VII was lower (P<0.05) than in group I. A weak negative correlation (r = -0.376) and a weak regression relationship (R^2=0.141) were found between CG intake and drumstick pH, indicating a mild acidifying effect of higher CG levels (Table 7). Overall, drumstick muscles had significantly higher pH values (P<0.001) than breast muscles (Table 8).

Differences (P<0.05) were observed among treatments in the L* (lightness) values of breast muscles (Table 8). For drumstick muscles, colour L* values were lower in groups III and VII than in group V (P<0.05) (Table 8). Colur a* (redness) and b* (yellowness)values of both breast and drumstick muscles were not affected (P>0.05) by treatments (Table 8). Overall, L*, a*, and b* parameters differed significantly between breast and drumstick muscles (P<0.001). A weak negative correlation (r= –0.334) and a weak regression relationship (R^2=0.139) were found between CG intake and the drumstick colour a* parameter, indicating that increased CG intake may reduce redness in drumstick muscles (Table 7).

Discussion

Increasing levels of CG produced limited yet notable effects on certain internal organ weights of broilers. No differences were observed among groups in terms of the relative weights of the gizzard, proventriculus, pancreas, liver, spleen, and abdominal fat (P>0.05). This result agrees with the results of Topal & Ozdogan (2013), Mousa et al. (2018), Sopian et al. (2020), Sehu et al. (2013), Farrapo et al. (2017), and Silva et al. (2012), who reported that the inclusion of CG or glycerol in diets did not significantly affect the development of these organs. These results suggest that increasing CG levels do not exert a direct stimulatory effect on lipogenesis.

In contrast, CG had a important effect on the relative heart weight. group VII was higher than that of the other groups (P<0.05). This result is consistent with the observations of Coşkun et al. (2007) and Topal & Ozdogan (2013), who indicated that higher CG levels may promote heart development. Moreover, the lowest relative heart weight recorded in the group I (P<0.05) supports the positive trend observed with increasing CG intake, which could be related to the involvement of glycerol in energy metabolism processes. An increasing CG level produced a positive tendency in liver weight. However, no difference was observed between the groups (P>0.05). Our result corroborates the findings of Sopian et al. (2020) and Mousa et al. (2018) reported that CG supplementation did not affect liver weight (P>0.05). The increase observed in the present study might be explained by the active participation of glycerol in hepatic energy conversion mechanisms, although differences among studies

could also be attributed to factors such as bird strain, CG inclusion level, and feeding duration.

Silva et al. (2019) reported that the inclusion of 2%, 4%, 6% refined glycerin in broiler diets did not affect intestinal weight or length. Contrary to their findings, the present study demonstrated that birds fed 10% CG throughout the trial (group VII) exhibited significantly higher ($P<0.05$) total intestinal weight (from gizzard outlet to cloaca) compared to the other groups (except Group VI, $P>0.05$). The consistent increase in intestinal weight with higher CG intake suggests that CG consumption may enhance overall intestinal development. These discrepancies among studies may stem from differences in strain, CG level, or feeding period, and might also be related to the utilization of CG as an energy source within the digestive tract. Although no differences were observed in pancreas weights ($P>0.05$), an increasing trend was detected with higher CG levels. Since pancreatic lipase hydrolyzes triglycerides into glycerol and fatty acids (Brody, 1994), and glycerol is readily soluble and absorbed into the portal circulation, this trend could indicate a potential contribution of CG to digestive enzyme activity.

In terms of DM content, the values obtained from the group without CG were higher than those of the groups fed 5% CG ($P<0.05$). This trend suggests that increasing glycerol levels may induce slight alterations in the water-holding capacity of muscle tissue. Furthermore, the DM content of breast muscles was found to be higher than that of drumstick muscles ($P<0.05$). Legawa et al. (2018) reported no significant difference ($P>0.05$) in breast muscle DM percentage between the group I and those fed diets containing 5% glycerin. Similarly, Topal & Ozdogan (2013) found no differences ($P>0.05$) in the DM content of drumstick muscles in broilers supplemented with 4% and 8% CG. Consistent with these findings, Silva et al. (2017) also observed no effect ($P>0.05$) on breast muscle DM in birds fed diets containing 0%, 2%, 4%, and 6% purified glycerin from days 22 to 42. In contrast, Silva et al. (2019) reported that increasing dietary glycerin levels (0%, 2%, 4%, and 6%) from days 1 to 42 significantly ($P<0.05$) and linearly decreased breast muscle DM content. The discrepancies between the current findings and those reported in the literature may be attributed to differences in the type and composition of CG used, overall diet formulation, dietary glycerol inclusion levels, and supplementation duration. Additionally, these variations may be explained by differences in muscle-type-specific water-binding capacities (Işık, 2008; Şekeroğlu and Diktaş, 2012; Urgnani et al., 2014).

Muscle CP content was found to be responsive to CG levels. The breast and drumstick muscles of groups I and II exhibited significantly lower protein contents than those of other groups ($P<0.05$). The linear increase in muscle protein with higher CG intake supports the potential role of glycerol in protein

sparing and amino acid metabolism. These findings contrast with those of Topal & Ozdogan (2013), Legawa et al. (2018), and Silva et al. (2017), who reported no effects (P>0.05), but agree with Silva et al. (2019), who observed a positive relationship between glycerin inclusion and muscle protein content.

No differences were found among groups in ether extract (EE) contents (P>0.05), though both drumstick and breast muscles showed a linear increase in fat content with rising CG levels (P<0.05), consistent with Silva et al. (2019). This tendency may be explained by the utilization of glycerol as a substrate in triglyceride synthesis, while the higher fat content of drumstick muscles could be related to their more oxidative nature (Legawa et al., 2018; Silva et al., 2019).

In this present study, pH values of breast and drumstick muscles were measured at 15 min and 24 h postmortem, and their mean values were considered as the ultimate pH. The pH values ranged from 5.98–6.39 in breast and 6.23–6.67 in drumstick muscles, remaining within physiological limits reported in the literature (Legawa et al., 2018; de Souza et al., 2020). Breast muscle pH was not significantly affected by CG levels (P>0.05), likely due to similar glycogen reserves at slaughter, as suggested by Haslinger et al. (2007). However, the drumstick pH was lower in the 10% CG group compared to the control (P<0.05), suggesting that higher glycerin inclusion might accelerate postmortem glycolysis and acidification. Variations may also be influenced by factors such as CG composition, feeding duration, strain, and bird age. These observations are consistent with those of Legawa et al. (2018), Garcia et al. (2018), and de Souza et al. (2020). It has been reported that raw breast meat appears light pink, whereas thigh and drumstick muscles are darker, reflecting their higher pH values and oxidative metabolism (Işık, 2008; Şekeroğlu and Diktaş, 2012; Urgnani et al., 2014). Moreover, pH plays a key role in muscle-to-meat conversion, affecting sensory characteristics such as color, water-holding capacity, and tenderness, and is influenced by both final pH and the rate of decline (Qiao et al., 2001).

Meat color is among the most important sensory attributes determining consumer perception of quality. Products with uniform and stable color are generally regarded as superior (Qiao et al., 2002). In this study, inclusion of up to 10% CG in broiler rations did not significantly affect breast and drumstick color parameters (L^*, a^*, b^*) (P>0.05) (except breast colour-L, P<0.05), indicating no adverse influence on visual meat quality. These results are consistent with those of de Souza et al. (2020), Urgnani et al. (2014), Sopian et al. (2020), and Silva et al. (2019), who reported no significant color changes with dietary CG supplementation. Meat color formation results from the selective absorption and reflection of light by myoglobin, muscle fibers, and structural proteins (Olivo et al., 2001). Postmortem muscle pH plays a decisive role in this process, as protein denaturation and water-holding capacity directly

affect surface light scattering (de Souza et al., 2020). Low pH (<5.8) values are associated with increased protein denaturation and pale color (Brossi et al., 2009), while higher pH (>6.2) produces darker meat due to reduced light reflection and enhanced water retention (Dransfield & Sosnicki, 1999). In this present study, the redness (a*) of drumstick muscles decreased significantly with increasing CG levels ($P<0.05$), whereas redness color of breast muscle remained unaffected ($P>0.05$). Garcia et al. (2018) similarly reported a linear reduction in thigh yellowness (b*) with increasing semi-purified glycerin levels, attributing this to a decrease in corn content, which is the major dietary source of xanthophylls and carotenoids (Scott and Eldridge, 2005). This observation aligns with the decreasing a* values recorded in drumstick muscles in the present study.

Other authors, such as Legawa et al. (2018), Urgnani et al. (2014), and Boonwong et al. (2018), also found no significant influence of CG inclusion on meat color parameters, whereas Faria et al. (2013) reported enhanced redness and hue changes with higher glycerin levels. Such discrepancies may arise from differences in glycerin purity, methanol content, source oil, or bird genotype. The numerical reductions in redness (a*) and yellowness (b*) values observed in both drumstick and breast muscles in this study are likely due to reduced pigment intake associated with lower corn inclusion. The carotenoid content of the oils used for glycerin production may also influence meat pigmentation. In this experiment, CG was derived from sunflower and canola oils, while corn oil served as the dietary fat source. Corn oil naturally contains carotenoids responsible for its yellow-orange color, though their concentrations vary depending on corn variety, cultivation conditions, and oil processing methods (Weber, 1987). Conversely, glycerin derived from palm oil, which is richer in lutein, has been shown to produce more intense yellow coloration in skin and meat (Faulks and Southon, 2005; Legawa et al., 2018). Therefore, the minor decreases in redness and yellowness observed in the present study are more likely related to differences in pigment intake and oil carotenoid composition rather than a direct effect of CG itself.

Overall, the inclusion of CG up to 10% in broiler rations did not adversely affect meat color quality. The minor variations detected appear to be associated with dietary composition and pigment availability. These findings indicate that CG may be used as a sustainable energy source in broiler nutrition without negatively influencing consumer-relevant quality traits such as meat color.

Conclusion

This present study demonstrated that dietary inclusion of CG up to 10% in broiler rations had no detrimental effects on internal organ development, muscle composition, or meat quality characteristics. The relative weights of

the spleen, gizzard, pancreas, proventriculus, and abdominal fat remained unaffected, while slight increases in heart, liver, and intestinal weights were observed at the highest CG level. Muscle chemical composition showed selective responses, with CP content increasing and ash remaining stable across treatments. Minor changes in fat deposition suggested that glycerol can be effectively utilized in lipid metabolism without impairing carcass quality. Meat pH and color parameters remained within physiological limits. Although the redness (a*) value of drumstick muscles decreased with increasing CG levels, breast meat color was not significantly influenced, indicating that CG inclusion did not compromise visual meat quality. These differences were likely related to variations in dietary pigment intake and the carotenoid profile of the oils used for CG production. Overall, the findings indicate that vegetable-derived CG can safely replace up to 10% of conventional energy sources in broiler diets. Its inclusion offers a sustainable and cost-effective strategy for poultry production, contributing to the valorization of biodiesel by-products and the advancement of circular bioeconomy practices.

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