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NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND
APPLIED SCIENCES DEPARTMENT OF ANIMAL
PRODUCTION AND TECHNOLOGIES



EFFECTS OF DIETARY BILE ACIDS AND LIPASE SUPPLEMENTATION ON
GROWTH PERFORMANCE, METABOLIC PROFILE, IMMUNITY, CARCASS
CHARACTERISTICS AND MEAT QUALITY OF BROILERS

TAREK DIAB

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TAREK DIAB

Master Thesis

Supervisor

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The study titled “**Effects of dietary bile acids and lipase supplementation on growth performance, metabolic profile, immunity, carcass characteristics and meat quality of broilers**” and presented by **TAREK DIAB** with the help of supervisor **Dr. Öğretim Üyesi Mabrouk ELSABAGH**, has been found as Master thesis by the jury at the Department of Animal Production and Technologies of Niğde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.

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THESIS CERTIFICATION

I hereby declare that all the information presented in this thesis has been gathered and compiled in accordance with scientific and academic principles. This study has been prepared while adhering to established academic guidelines and regulations. Furthermore, any assistance or resources utilized during the preparation of this thesis have been appropriately acknowledged wherever necessary.

Tarek DIAB



ABSTRACT

EFFECTS OF DIETARY BILE ACIDS AND LIPASE SUPPLEMENTATION ON GROWTH PERFORMANCE, METABOLIC PROFILE, IMMUNITY, CARCASS CHARACTERISTICS AND MEAT QUALITY OF BROILERS

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Bile acids (BAs) have potential as dietary supplements to enhance broiler health and performance, yet their effects remain inconsistent, and their synergy with lipase is not well explored. This study evaluated BAs alone and in combination with lipase as alternatives to oxytetracycline (OTC) in broilers. A total of 240 Ross 308 broilers were assigned to four groups: Control, OTC (100 mg/kg), BAs (0.27–0.25 g/kg), and BAs-L (BAs + 0.18 g/kg lipase). Growth performance, immune response, lipid metabolism, liver and kidney function, digestive enzyme activity, carcass traits, and cecal bacterial counts were analyzed. Results showed no significant differences between OTC and control groups for most parameters. However, OTC reduced feed intake, IgG, ALT, and total bacterial counts. Broilers fed BAs, alone or with lipase, had the highest weight gain, improved FCR, PER, EER, and EPEF, and lower abdominal fat. BAs groups also showed reduced serum lipids, liver enzyme activities, and uric acid while increasing IgG and digestive enzyme activity. BAs with lipase improved early growth but did not enhance all performance metrics compared to BAs alone. These findings confirm BAs as a viable alternative to OTC, warranting further research on optimal doses.

Keywords: broilers, bile acid, lipase, antibiotic growth promoters, performance, health

ÖZET

RASYONA SAFRA ASİTLERİ VE LİPAZ İLAVESİNİN BROYLERLERİN BÜYÜME PERFORMANSI, METABOLİK PROFİLİ, BAĞIŞIKLIK, KARKAS ÖZELLİKLERİ VE ET KALİTESİ ÜZERİNE ETKİLERİ

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Bil Safra asitleri (BAs), broiler sağlığı ve performansını artırmak için diyet takviyesi olarak potansiyel taşımaktadır, ancak etkileri tutarsızdır ve lipaz ile sinerjisi yeterince araştırılmamıştır. Bu çalışma, BAs'ı tek başına ve lipaz ile kombinasyon halinde oksitetrasiklin (OTC) alternatifleri olarak değerlendirdi. Toplam 240 Ross 308 broiler, dört gruba ayrıldı: Kontrol, OTC (100 mg/kg), BAs (0,27–0,25 g/kg) ve BAs-L (BAs + 0,18 g/kg lipaz). Büyüme performansı, bağışıklık yanıtı, lipid metabolizması, karaciğer ve böbrek fonksiyonları, sindirim enzimi aktivitesi, karkas özellikleri ve çekum bakteri sayımları analiz edildi. Sonuçlar, OTC ve kontrol grupları arasında çoğu parametre açısından anlamlı bir fark göstermedi. Ancak, OTC yem alımını, IgG, ALT ve toplam bakteri sayısını azalttı. BAs ile beslenen broilerler, tek başına veya lipaz ile birlikte, en yüksek kilo alımı, iyileştirilmiş FCR, PER, EER ve EPEF ile daha düşük abdominal yağ gösterdi. BAs gruplarında ayrıca düşük serum lipid seviyeleri, karaciğer enzim aktiviteleri ve ürik asit gözlenirken, IgG ve sindirim enzimi aktivitesi arttı. Lipaz ile birlikte BAs erken büyümeyi iyileştirdi ancak BAs'a kıyasla tüm performans ölçütlerini artırmadı. Bu bulgular, BAs'ın OTC'ye alternatif olarak etkinliğini doğrulamakta olup, optimal dozların belirlenmesi için daha fazla araştırmaya ihtiyaç duyulduğunu göstermektedir.

Anahtar Kelimeler: broilerler, bil asitleri, lipaz, antibiyotik büyüme promotörleri, performans, sağlık.

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SYMBOLS AND ABBREVIATIONS

Abbreviations	Descriptions
OTC	Oxytetracycline hydrochloride 99.8%
BAs	Bile acids
L-BAs	Bile acids plus lipase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BBSP	Bovine Bile Salts Powder
BW	Body Weight
CA	Cholic Acid
CDCA	Chenodeoxycholic Acid
CP	Crude Protein
DAA	Digestible Amino Acids
DBA	Desiccated Bile Acid
DBWG	Daily Body Weight Gain
DCA	Deoxycholic
AGPs	Antibiotic growth promoter
FCR	Food Conversion Ratio
G	Gram
HDL	High-Density Lipoprotein
HLB	High Hydrophilic-To-Lipophilic Balance
LCA	Lithocholic Acid
LDL	Low-Density Lipoprotein
EPEF	European production efficiency factor
PER	Protein efficiency ratio
EER	Energy efficiency ratio
NE	Necrotic Enteritis
P	Probability

PEGR	Polyethylene Glycol Ricinoleate
RBO	Rice Bran Oil
Tau	Taurine
Vit	Vitamin
WG	Weight Gain
YL	Yarrowia Lipolytica



CHAPTER I

INTRODUCTION

1.1 Aims and Objectives

Animal protein is a crucial source of essential amino acids necessary for various bodily functions. Broiler meat is an affordable and efficient animal protein source that can greatly contribute to food security and meet the global rising demand for animal-based protein sources (Birhanu et al., 2023). Sustainable broiler meat production is influenced by the birds' genetics, feed costs, effective farm management practices and meat prices, (Bist et al., 2024). Improving nutrient utilization and reducing production costs while saving the environment are key components of sustainable broiler meat production (Bist et al., 2024; Haque et al., 2020). Genetic advancements in broiler strains have greatly raised their growth potential, increasing the demand for higher energy intake. Energy density in broilers' diets is augmented by feeding the plant-derived oils due to their higher unsaturated fats content that are efficiently utilized by poultry (Baião and Lara, 2005; Tancharoenrat, 2012). However, young chicks have a limited capacity to digest and absorb fats due probably to their immature digestive system, inefficient enzymatic activity, and insufficient bile acids (BAs) production which are crucial for emulsifying fats and facilitating their digestion and absorption. Endogenous BAs are synthesized in the liver from cholesterol, stored in the gallbladder, and released through the bile flow into the intestine during digestion (Begley et al., 2005; Perrone et al., 2010).

Dietary supplementation of exogenous BAs has been shown to improve fat digestion and absorption, contributing to overall improvement in nutrient utilization and growth performance in broilers (Geng et al., 2022). The BAs' actions are mediated by enhancing fat digestibility, stimulating digestive enzyme activity, and positively affecting hepatic and serum lipid profiles (Arshad et al., 2021; Hanif et al., 2024; Hu et al., 2024a).

Additionally, BAs act as signaling molecules that activate the farnesoid X receptor (FXR) and the G-protein-coupled bile acid receptor 1 (TGR5). These receptors regulate insulin sensitivity and hepatic glucose production, leading to improved glucose tolerance and reducing the risk of metabolic disorders (Pols et al., 2011; Jia et al., 2018).

Antibiotic growth promoters (AGPs) have been intensively used in poultry production to enhance their performance and feed efficiency. Also, AGPs increase productivity and reduce production costs by promoting gut health and reducing infections. However, significant concerns are rising regarding the use of AGPs due to their implications for antibiotic resistance and overall poultry health (Van Boeckel et al., 2015; Paul et al., 2022). The unwise use of AGPs in livestock has contributed to the emergence of antibiotic-resistant bacterial strains, posing serious challenges for treating infections in both humans and animals (Mathew et al., 2007; Bava et al., 2024). Antimicrobial resistance (AMR) is a serious global health challenge because AMR genes can circulate among animals, humans and the environment through food chains (Marshall and Levy, 2011; Ferri et al., 2017). Accordingly, there is an increasing interest in exploring non-antibiotic substitutes in broiler meat production to fight the emerging threat of AMR. It has been proposed that BAs are a potential alternative to AGPs in poultry production as BAs possess antimicrobial properties and promote gut homeostasis and immunity (Jia and Dong 2024; Liu et al., 2024a; Yin et al., 2021a; Zhang et al., 2023). Also, BAs have been shown to enhance defense mechanisms against endotoxins and maintain intestinal barrier integrity, thereby reducing systemic inflammation and improving health outcomes (Kachrimanidou and Tsintarakis, 2020; Cai et al., 2022).

Based on the above context, BAs play a crucial role in enhancing the emulsification, digestion, and absorption of dietary fats in poultry (Alzawqari et al., 2023) especially in young chickens where digestive systems are still developing and the capacity to produce lipase and BAs adequate for lipid digestion is not fully functioning (Krogdahl 1985).

Thus, dietary administration of exogenous lipases and BAs to broilers can address these limitations by improving fat utilization (Arshad et al., 2020). Various studies indicated that BAs and lipases greatly enhanced broilers' nutrient utilization and growth performance (Ahmad et al., 2023; Arshad et al., 2021). Recently, microbial lipases are increasingly recognized for their effectiveness as hydrolysis catalysts in the poultry industry compared to pancreatic lipases derived from animal sources (Alzawqari et al., 2023; Arshad et al., 2021). Thus, both BAs and microbial lipases contribute positively to feed efficiency in poultry by increasing digestive enzyme activity and improving nutrient digestion and absorption, thereby promoting better overall growth performance, especially in young chicks where BAs secretion and recycling and lipase activity may be

limited (Arshad et al., 2020; Shoaib et al., 2021; Alzawqari et al., 2011; Neto et al., 2011; Alzawqari et al., 2016; Rezaeipour et al., 2016). Dietary supplementation of BAs (0.004% to 0.25%) and lipase (0.01% to 0.1%) has been shown to improve fat digestibility and enhance overall growth performance and nutrient utilization in broilers (Abd El-Aziz et al., 2025; Arshad et al., 2021).

In summary, exogenous BAs and lipases are essential for mitigating limitations in the digestive physiology of young chickens. Several studies addressed the benefits of exogenous BAs and lipase supplementation separately in broiler diets, but the synergistic effects of their combined administration necessitate further exploration. Also, the potential of BAs as alternatives to AGPs remains unclear. Furthermore, the inconsistent results regarding the efficacy of the BAs and lipase supplementation in broilers' diets highlight a knowledge gap that necessitates more investigations (Abd El-Aziz et al., 2025; Shoaib et al., 2023).

Therefore, this study evaluated the effects of dietary supplementation of exogenous BAs, either alone or in combination with lipase, on growth performance, feed efficiency, metabolic profile, liver health, immune response, and carcass characteristics in broilers. Also, these effects of BAs were compared to those of the antibiotic growth promoter oxytetracycline (OTC) to explore their potential as substitutes for AGPs. Co-administration of BAs and lipase could optimize feed efficiency, reduce fat deposition, and promote the growth performance of broilers. Also, BAs can positively modulate gut microbiota and enhance the overall metabolic health of broilers, thereby reducing antibiotic use in poultry production. Improving feed efficiency and animal health is crucial for addressing food security and sustainability challenges.

CHAPTER II

REVIEW OF LITERATURE

2.1 Endogenous Bile Acids

Fat is a high-calorie, energy-dense component in broilers diets. The chicken's ability to utilize dietary fat improves as they grow older. However, fat utilization in young chickens is often limited, primarily due to inadequate BAs secretion and recycling rather than a lipase activity insufficiency (Alzawqari et al., 2023; Shoaib et al., 2021). It has been proposed that young chickens have a lower capacity to synthesize and recycle BAs until their digestive systems mature, approximately around 10–14 days of age (Leeson and Summers, 2005; Noy and Sklan 1997; Tancharoenrat et al., 2013). Thus, the broiler's ability to utilize dietary fats improves as its digestive system develops, leading to better utilization of fat and lipid-soluble nutrients (Maisonnier et al., 2003).

The production and release of pancreatic lipase, essential for breaking down triglycerides (TG) into absorbable forms, are also enhanced by the presence of BAs, which are released in response to cholecystokinin (CCK). CCK is a gut hormone secreted from I cells in the intestinal mucosa, particularly in the duodenum, in response to the presence of dietary fat in the intestinal lumen (Brownlee et al., 2010; Otsuki 2000). While pancreatic lipase is essential for breaking down TG into absorbable forms, the formation of micelles, a process dependent on the presence and concentration of BAs, ultimately determines how effectively these lipids can be absorbed in the intestine. Consequently, one of the primary limitations in fat utilization is not the hydrolysis of TG by lipase, but the efficient micelle formation, which ensures that fat molecules are properly emulsified and available for absorption in the intestines (Tancharoenrat, 2012; Tancharoenrat et al., 2013).

Endogenous BAs are steroid acids that are primarily found in the bile of mammals. They are classified as primary or secondary BAs based on their synthesis location and metabolic processes. Primary BAs are synthesized in the hepatic peroxisomes (Ferdinandusse et al., 2009) where cholesterol is converted into BAs through several enzymatic reactions (Chiang, 2009). The initial stage of synthesis is facilitated by cholesterol 7 α -hydroxylase (CYP7A1). The expression of the gene responsible for

encoding CYP7A1 has been reported to be inhibited by several factors, which include, but are not limited to, insulin, cytokines, protein kinase C activators, steroid hormones, and BAs (Chiang and Ferrell, 2020). They are amphipathic molecules, i.e., having both hydrophilic and hydrophobic properties, allowing them to interact with fats and water effectively (Hanafi et al., 2018). In poultry, the predominant primary BAs are cholic acid (CA) and chenodeoxycholic acid (CDCA), which are conjugated with glycine or taurine in the intestinal tract to form conjugated BAs like glycocholic (GCA) and taurocholic (TCA) acids (Leeson and Summers, 2005). The emulsifying properties of BAs are strengthened by this conjugation, facilitating the intestinal digestion and absorption of dietary fats and fat-soluble nutrients. The combination of conjugated BAs with sodium or potassium ions further enhances BAs solubility and functionality, leading to more effective emulsification of dietary fats (Hofmann, 1999).

On the other hand, secondary BAs primarily include deoxycholic acid (DCA) and lithocholic acid (LCA) which are produced through deconjugation and dehydroxylation of primary BAs by gut microbiota (Bansal et al., 2021; Hofmann 2009; Mertens et al., 2017). It has been indicated that gut microbiota greatly affects the composition and enterohepatic circulation of BAs and modulates their receptors, such as FXR and TGR5, involved in metabolic signaling (Van Beilen et al., 2020). For example, chickens raised in sterile environments, with limited gut microflora, exhibit higher levels of intestinal conjugated BAs and lower levels of unconjugated BAs compared to conventionally raised chickens, highlighting the crucial role of gut microbiota in modulating BAs composition (Maisonnier et al., 2003). Interestingly, these bacterial transformations begin in early life as 16 distinct BAs have been identified in meconium (Hofmann 1999; Jönsson et al., 1995). Although these bacterial transformations are essential for maintaining a healthy gut microbiota, they may decrease fat utilization due to the lower emulsification efficiency of these secondary BAs compared to the primary ones. Thus, the early-life bacterial transformation of primary BAs highlights the importance of maintaining adequate levels of effective BAs during this critical growth period. Accordingly, supplementation of exogenous BAs can compensate for the effects of the deconjugation of endogenous BAs, thereby ensuring that young chickens have adequate access to primary (conjugated) BAs essential for optimal fat digestion and overall nutrient utilization.

After aiding in digestion, primary endogenous BAs are recycled through enterohepatic circulation, where they are reabsorbed in the ileum and transported back to the liver to be reused for further digestion. Approximately 95% of primary BAs are reabsorbed in the ileum, while the remaining 5% enter the colon where gut microbiota transforms them into secondary BAs that may be partially reabsorbed or excreted (Hofmann, 2009; Mertens et al., 2017; Vlahcevic et al., 1996; Dawson and Karpen, 2015). This recycling process serves to conserve BAs production and maintain a constant pool of BAs within the body. Because of its hydrophobic nature, LCA is poorly recycled into the enterohepatic circulation, while DCA is more readily reabsorbed in the colon and accumulates in the BAs pool. As a result, the BAs pool is predominantly composed of CA, CDCA, and DCA, which together account for roughly 40%, 40%, and 20% of the total BAs pool, respectively (Ashby et al., 2018; El-Katcha et al., 2019; Larabi et al., 2023).

Beyond their role in enhancing the utilization of fat and lipid-soluble nutrients, BAs act as signaling molecules activating specific receptors, such as FXR and TGR5, that play crucial roles in regulating glucose and lipid metabolism, highlighting the endocrine functions of BAs (Chiang, 2009; Pols et al., 2011; Vitek and Haluzik 2016). Moreover, BAs exert antimicrobial actions and maintain gut barrier integrity (Alzawqari et al., 2023) and can protect against gut injury or infection (Perrone et al., 2010). Previous research demonstrated that taurodeoxycholic acid (TDCA) supports cell turnover and maintains intestinal barrier integrity (Laukens et al., 2016). Additionally, TDCA alleviated mucosal damage and enhanced survival rates in cases of lipopolysaccharide-induced intestinal injury in vitro (Wu et al., 2019). Moreover, dietary BAs have strengthened gut integrity and improved survival in murine models (Perrone et al., 2010). Therefore, regulation of BAs synthesis and administration is critical for maintaining proper fat utilization and overall metabolic health (Chiang et al., 2009; Hofmann, 1999).

There is an increasing interest in alternatives to AGPs in animal nutrition due to the rising concern of AMR. Due to their ability to modulate gut microbiota and maintain microbial balance, BAs may have the potential as alternatives to AGPs. Previous studies indicated that BAs supplementation can improve the growth performance and nutrient utilization of livestock by increasing beneficial microbes and inhibiting pathogenic bacteria, thereby reducing the need for AGPs (Van Beilen et al., 2020; Jia and Dong, 2024). This approach can promote a healthier gut microbiota, reducing dependency on antibiotics and lowering

the risk of AMR. Thus, the dynamic interactions between BAs and gut microbiota highlight the role of BAs not only in nutrient utilization but also in regulating gut microbiota homeostasis, which affects overall animal well-being and health (Cai et al., 2022).

The types and concentrations of BAs produced can vary among species, and their production is influenced by diet, health status, and metabolic needs. For example, dietary fat levels can impact BAs synthesis and secretion, as animals adjust their BAs production to optimize fat digestion (Alzawqari et al., 2011; Hofmann et al., 1999). This adaptability underscores the importance of BAs not only in digestion but also in broader metabolic regulation. Nonetheless, the dysregulation of BAs production or metabolism is associated with several intestinal and hepatic dysfunctions, making it a significant focus of ongoing research in the biomedical field (Wang et al., 2024a).

2.2 Exogenous Bile Acids

Exogenous BAs are commercially available and increasingly used as natural emulsifiers in animal feeds (Abdel-Tawwab et al., 2023; Arshad et al., 2021; Bansal et al., 2021; Hasibuan et al., 2025; Yin et al., 2021a). Their supplementation not only enhances fat digestion but also contributes to improved growth performance and overall health in animals, especially in cases of immature digestive systems (Sun et al., 2023), liver dysfunction (Hofmann and Hagey, 2008), intestinal inflammation, nutrient-dense or high-fat diets (Chiang, 2013), or periods of high growth demand (Yin et al., 2021b). Recently, BAs have emerged as potential alternatives to AGPs in animal production. Their ability to modulate gut microbiota, enhance immune responses, and improve antioxidant status contribute to enhanced resistance against infections, which consequently lowers the dependence on antibiotics (Hu et al., 2024b; Ridlon et al., 2014).

2.2.1 Effects of Exogenous BAs Supplementation on Broilers' Nutrition and Health

Exogenous BAs, primarily derived from animal sources such as ox and porcine bile, as well as microbial transformations in the gut, have been shown to significantly influence various performance and health metrics in broiler chickens. The following section will summarize key findings regarding the effects of exogenous BAs from several studies.

Maisonnier et al. (2003) evaluated the effects of sodium taurocholate (Na-TCA) at 0 or 0.3% in diets of broiler strain Y33 with conventional or limited gut microbiota. Supplementing broiler diets with Na-TCA increased fat digestibility with a more pronounced effect in conventional birds compared to those with limited microflora, due to the low basal level of intestinal BAs in the conventional chickens. Conventional birds exhibited higher levels of intestinal conjugated BAs and lower levels of intestinal unconjugated BAs, highlighting the crucial role of gut microflora in modulating BAs composition.

Parsaie et al. (2007) evaluated the effects of adding CA (500 mg/kg) versus the antibiotic growth promoter virginiamycin (200 mg/kg) on the performance and gut morphology of Ross 385 chicks fed wheat-based diets over 18 days. Both treatments increased feed intake (FI) and daily body weight gain (BWG) but had no significant impact on the feed conversion ratio (FCR). Notably, CA supplementation reduced liver and caeca weight, as well as the length of the duodenum, jejunum, ileum, and overall small intestine. Additionally, CA addition decreased both the height and width of intestinal villi compared to virginiamycin.

Alzawqari et al. (2011) investigated the effects of dietary administration of ox BAs on growth performance, blood lipid profile, and gut health in Ross 308 male chicks fed high-fat diets (5% tallow). Three levels of dietary ox BAs (0%, 0.25%, and 0.50%) were included during the starter (7-21 days) and grower (22-42 days) phases. Results indicated that ox BAs supplementation significantly improved BWG and FCR without affecting FI. While blood lipid profiles remained unaffected, the supplementation notably enhanced fat digestibility and gut morphology (increased crypt depth and villus dimensions in the jejunum and ileum).

Rezaeipour et al. (2016) investigated the effects of bovine bile salt powder (BBSP), obtained from bovine gallbladders at a slaughterhouse and oven-dried at 55°C for 24 hours, on growth performance, carcass traits, and metabolic profile of one-day-old mixed-sex broiler chickens. BBSP was administered at two levels (0 or 2.5 g/kg) in conjunction with two levels of soybean oil (low: 1.5, 3, and 4.5%; high: 3, 4.5, and 6%, for starter,

grower, and finisher stages, respectively) over a 42-day feeding period. The study revealed that BBSP had no significant impact on growth performance parameters, serum metabolite levels (glucose, cholesterol, TG, and high-density lipoprotein, HDL), or liver enzyme activities (alanine aminotransferase, ALT and aspartate aminotransferase, AST). However, BBSP increased thigh weight compared to the control group.

Alzawqari et al. (2016) studied the impact of adding 80% desiccated bile acid (DBA), sourced from the gallbladders of oxen at a slaughterhouse, on broiler chickens fed corn-based diets. Three levels of DBA (0.0%, 0.25%, and 0.50%) with 3 levels of taurine (0.0, 0.25 and 0.50%) were tested to assess their effects on performance, digestion, serum lipid profile, and carcass characteristics. They found that supplementing the feed with 0.50% DBA significantly improved FI, BWG, and FCR for the grower and the cumulative periods compared to 0.0 and 0.25%. Additionally, it enhanced fat digestion, increased serum cholesterol, TG, HDL, and low-density lipoprotein (LDL), and reduced liver and small intestinal weight. However, it did not affect carcass dressing percentage or ileal viscosity while taurine supplementation increased bursa weight. In their later experiment, Alzawqari et al. (2023) assessed the impact of the same BAs levels (0.0%, 0.25%, and 0.50%) with three levels of xylanase (0 %, 0.05 %, and 0.10 %) on growth, fat absorption, blood lipid profiles, and intestinal viscosity in male broilers fed wheat-based diets over 35 days (7–42 d). The results showed no changes in FI, BWG, and FCR but BAs adding significantly improved fat digestibility. At a 0.5% BAs level, there was a marked increase in cholesterol and LDL levels. Additionally, xylanase effectively reduced intestinal viscosity by breaking down arabinoxylan, thus enhancing fat utilization in wheat-based diets.

San Tan et al. (2016) investigated the impact of supplementing male Cobb broiler chicks' diets with polyethylene glycol ricinoleate (PEGR), a commercial exogenous emulsifier with a high hydrophilic-to-lipophilic balance, on fat digestibility. The experimental diets consisted of standard broiler starter and grower diets for 5 weeks, using rice bran oil as the fat source and supplementing with 0.05% emulsifier. The addition of PEGR resulted in higher apparent metabolizable energy, improved FI, and BWG but there was no significant difference in fat digestibility between the control and emulsifier-supplemented

groups. The FCR was similar to the control group, except in week 5 where it was higher with PEGR supplementation.

Lai et al. (2018a) evaluated lipid metabolism and lipase activity in Arbor Acres male broiler chicks fed BAs at 0, 40, 60, or 80 mg/kg diet, respectively, over 42 days. The BAs were extracted from pig bile and comprised 77.2% combined hyocholic acid and hyodeoxycholic acid plus 19.9% CDCA. BAs supplementation increased BWG and improved FCR but did not affect FI. Broilers fed diets supplemented with 60 and 80 mg/kg BAs showed a higher percentage of carcass dressing and thigh muscle and a lower percentage of abdominal fat. Additionally, they exhibited higher activity of duodenal lipase and lipoprotein lipase, and lower activity of hormone-sensitive lipase. There was no change in serum lipid metabolites (TG, LDL, HDL) among treatments.

Ge et al. (2019) used the same BAs as Lai et al., 2018 to assess their effect on hepatic lipid metabolism and carcass quality of Arbor Acres broilers fed a high-energy diet over 42 days (1-21 days for starter and 22-42 days for finisher). Two BAs levels (60 mg/kg for starter and 80 mg/kg for finisher) and two energy levels: basal energy (2,940 kcal/kg for starter; 3,110 kcal/kg for finisher) and high energy (3,030 kcal/kg for starter and 3,200 kcal/kg for finisher) diets, with energy provided by lard, were used. Supplementing feed with BAs improved BWG and FCR without affecting FI. It also increased breast muscle ratio and ameliorated hepatic and abdominal fat accumulation caused by feeding high-energy diets. Additionally, BAs supplementation decreased serum TG and increased hepatic lipoprotein lipase activity, decreased the expression of fatty acid synthase (FAS) and increased the expression of carnitine palmitoyltransferase 1.

Mohamed et al. (2020) evaluated the effects of a 70% bile salt supplement (composed of 46.9% CA, 17.8% DCA, and 4.06% CDCA) at three levels (0.5, 1, and 1.5 ml /kg diet) on performance and health parameters of Ross 308 broiler chickens over 42 days. Supplementation decreased FI but increased BWG leading to improved FCR. It also enhanced nutrient digestion, immune response (higher immunoglobulin G, IgG), antioxidant capacity (higher superoxide dismutase, lower malondialdehyde), and digestive enzyme activity (amylase, protease, and lipase) but increased total cholesterol

(TC) and TG. Additionally, bile salt supplementation reduced gut harmful bacteria (*E. coli*, *Salmonella*).

Bansal et al. (2021) investigated the efficacy of DCA, a secondary bile acid, in mitigating necrotic enteritis (NE) in broiler chickens induced by *Eimeria maxima* and *Clostridium perfringens* infection. Supplementation of DCA at three levels (0.8, 1.0, and 1.5 g/kg) in the diet of day-old chicks reduced NE-induced body weight loss, lowered inflammatory gene expression and cell apoptosis in the intestines, decreased *C. perfringens* colonization, and improved ileal histopathology scores in a dose-dependent manner. These findings suggest that DCA may be a potential antimicrobial alternative to combat intestinal diseases in poultry by alleviating NE-induced inflammation and bile acid depletion.

Previous studies indicated that BAs can be effective dietary supplements during heat stress in poultry. Yin et al. (2021a) indicated that BAs (200 mg/kg diet) from pig bile restored gut microbiota homeostasis, maintained villus structure and decreased gut inflammatory response in broilers under heat stress. Also, BAs (200 mg/kg diet) enhanced the hepatic antioxidant capacity and decreased the expression of lipogenic genes and fat deposition in the liver of heat-stressed broilers (Yin et al., 2021a).

Hussien et al. (2022) investigated the effects of supplementing BAs (Runeon, 200 g/ton) in the diet of Ross-308 broiler chickens fed a reduced energy diet (30 kcal/kg lower) for 31 days. Supplementation significantly improved growth performance (BWG, FCR, and European production efficiency factor, EPEF), carcass characteristics (increased dressing percentage and breast, thigh, and drumstick meat yield, with decreased abdominal fat), meat quality (reduced moisture and fat content, with slight increases in crude protein and ash), metabolic profile (lowered serum cholesterol, TG, HDL, and LDL and elevated total serum protein), and intestinal lipase activity.

In comparison to tylosin, an antibiotic growth promoter, Zhang et al. (2022) indicated that BAs addition at 4.5 mg/kg significantly promoted hepatic lipolysis and reduced lipogenesis as was confirmed by regulating the expression of specific genes, respectively,

compared to feeding tylosin at 2 mg/kg in yellow-feathered broilers, suggesting that BAs are a potential alternative to tylosin in broilers production.

Research indicated a promising role of BAs in the protection against mycotoxins in broilers. Chen et al. (2023) indicated that feeding BAs (250 mg/kg diet) derived from pig bile was effective in protecting the liver against aflatoxin B1 as indicated by activating the genes involved in aflatoxin detoxification and transformation in broilers.

Wang et al. (2024b) assessed the impact of dietary supplementation of BAs (used in Lai et al., 2018b) on the hepatic lipid profile and cecal microbiota of Arbor Acres broiler chicks. BAs were added at 60 mg/kg to either low-fat (2.22% from days 1 to 21 and 4.79% from days 22 to 42) or high-fat (5.40% from days 1 to 21 and 7.65% from days 22 to 42) diets. Results revealed that BAs supplementation significantly reduced TG levels in both plasma and liver and lowered FAS expression in the liver, suggesting reduced fat accumulation. In the low-fat group, BAs supplementation improved both BAs concentration and the ratio of non-12-OH (taurochenodeoxycholic acid, TCDCA, and CDCA) to 12-OH bile acids (taurocholic acid, TCA, and CA) in the liver while enhanced the abundance of Akkermansia in the cecum. Conversely, in the high-fat group, BAs supplementation decreased overall BAs levels but increased CDCA and CA concentrations in the liver. Simultaneously, it decreased the relative abundance of Bacteroides and increased levels of Bifidobacterium, Escherichia, and Lactobacillus in the cecum. The results indicated that changes in liver lipid profiles were associated with alterations in microbiota-BAs profiles with BAs supplementation to diets with different fat levels. However, the authors suggested that varying fiber contents in low (2.3% at 1-3 weeks, and 2.1% at 4-6 weeks) and high (3% at 1-3 weeks, and 2.8% at 4-6 weeks) fat diets might contribute to changes in gut microbiota.

Regarding the antibacterial effects of BAs, it was indicated that BAs (350 mg/kg diet) were effective in protecting the intestinal barrier and reversing gut dysbiosis caused by Salmonella Typhimurium infection compared to antibiotic treatment in broilers (Hu et al., 2024a). Also, BAs increased the abundance of beneficial bacteria and decreased that of harmful ones in broilers infected with salmonella.

As mentioned above regarding the protective effects of BAs against heat stress, the anti-stress effects of BAs were further confirmed in broiler chickens under corticosterone stress. Liu et al. (2024b) indicated that supplementing BAs (250 mg/kg diet) reduced TG and fatty acid synthesis as well as fatty acid transport in the liver of broilers under corticosterone stress.

Recently, Hasibuan et al. (2025) evaluated the effects of varying levels (200-600 mg/kg diet) of bovine bile extract (BBE), derived from the gallbladder of freshly slaughtered cattle, and commercial BAs on broiler performance, carcass characteristics, meat quality, serum lipid profile, and fat digestibility in broilers fed 8% fat from day 8 to days 32 of age. The supplementation with either additive increased growth performance, decreased meat content of fat and increased protein content, and improved fat digestibility but there was no change in serum TC and TG, or carcass yield in broilers fed a high-fat diet. Yet, a 600 mg/kg of BBE or 200 mg/kg diet of commercial BAS was more economical in terms of return over the cost of chicks and feeds.

2.3 Lipase Enzymes

2.3.1 Endogenous Lipase Enzymes

Lipases, classified as triacylglycerol acyl hydrolases (EC 3.1.1.3), are vital enzymes that catalyze the hydrolysis of TG into glycerol and free fatty acids. These enzymes are primarily produced in the pancreas and secreted into the intestine, facilitating the breakdown of dietary fats. In young birds, particularly those with immature digestive systems, there is often a deficiency in endogenous BAs and pancreatic lipase activity, leading to inefficient fat utilization (Krogdahl, 1985; Ravindran et al., 2016; Shoaib et al., 2023). Research indicates that supplementing diets with exogenous lipases can significantly improve fat utilization and overall growth performance in young birds (Ahmad et al., 2023; Al-Marzooqi and Leeson 2000; Fernandes et al., 2023; Hu et al., 2018a; Shabani et al., 2021).

Mammalian lipase derived from porcine sources and microbial lipases sourced from fungi and bacteria are the primary sources of the commercially available exogenous lipases

used in poultry nutrition (Kermanshahi, et al., 1998; Ravindran et al., 2016). Microbial lipases originating from *Rhizopus arrhizus*, *Aspergillus niger*, and various *Pseudomonas* species, are recognized for their stability and efficiency in fat digestion (Kumar et al., 2023; Lima et al., 2019). Fungal lipases derived from *Aspergillus niger* exhibit significant stability in acidic conditions that are comparable to the pH of the glandular stomach. Research indicates that the optimal pH for *Aspergillus niger* lipase is around 5, while lipase from *Chromobacterium viscosum* lipase performs best at pH levels ranging from 6 to 8 (Kermanshahi, et al., 1998; Zhu et al., 2020). Also, research indicates that many lipases experience a decline in activity after 30 minutes at 40°C and pH 7, except for lipase from *Aspergillus niger*, which maintains its stability under these conditions (Chandra et al., 2020; Kumar et al., 2023; Lima et al., 2019). Furthermore, Wang et al. (2018) have shown that lipase from *Yarrowia lipolytica* (YL) exhibits enhanced stability under acidic conditions when combined with spray-dried skim milk powder and starch, allowing lipase to maintain a significant activity even in the presence of proteases and bile salts. Among various fungal lipases, YL lipase supernatant has been noted for its superior stability at low pH compared to other strains like *Rhizopus oryzae*, *Candida rugosa*, and *Thermomyces lanuginosus* (Wang et al., 2018).

Yet, studies on supplemental lipase in broilers are limited and some results are contradictory. Some studies have reported no significant effects on growth performance or nutrient digestibility with lipase supplementation (Ahmad et al., 2023; Fernandes et al., 2023a; Meng et al., 2004; Oliveira et al., 2019), proposing that insufficient pancreatic lipase production might not be a primary factor affecting fat digestion in young broilers (Ravindran et al., 2016).

While inconsistency in findings may stem from differences in experimental design, including diet composition, levels and origin of lipase, and the age of the broilers, further investigations are warranted to establish clearer guidelines for the effective use of lipase in poultry nutrition.

2.3.2 Effects of Exogenous Lipase Supplementation on Broilers' Nutrition and Health

Research on exogenous lipase supplementation in poultry diets remains limited, with inconsistent findings on broilers' fat digestion and overall growth performance. Some studies identified exogenous lipases as beneficial for enhancing feed efficiency, particularly during early life stages and/or energy-restricted diets (Al-Marzooqi and Leeson 2000; Hu et al., 2018b; Shoaib et al., 2021; Wang et al., 2018). On the other hand, several studies have found no significant impact of these enzymes on nutrient utilization, growth performance, serum lipid profiles, or carcass characteristics, especially when they are administered alone (Ahmad et al., 2023; Arshad et al., 2020; Fernandes et al., 2023; Meng et al., 2004). These studies reported factors including diet composition, bird characteristics, fat sources, bile salts, as well as lipase source and purity to affect lipase effectiveness.

Al-Marzooqi and Leeson (2000), assessed the anorexic effect of pancreatic lipase (0, 0.375, 0.750, or 1.125%) in male broiler chicks over 3 weeks (d 1-21). The BWG and FI decreased while liver weight increased with increasing levels of pancreatic lipase without affecting FCR. However, after d 21 when lipase was no longer used, lipase had no effects on BWG, FI and liver weight while enhancing FCR, implying that lipase might have no long-term negative carry-over effects but voluntarily reduced FI, or lipase might be contaminated with gut hormones affecting appetite, as authors indicated (Al-Marzooqi and Leeson, 2000).

Meng et al. (2004) investigated supplementing lipase (0.0 or 0.2 g/kg of diet) on growth performance and nutrient digestion of male broilers fed varying fat type (50 g/kg diet from beef tallow or canola oil) and carbohydrase addition (0 or 0.4 g/kg diet) to a wheat-based diet from 5-18 days old. Lipase did not affect chicken performance and nutrient utilization, indicating that suboptimal lipase activity rather than insufficient pancreatic lipase production might be a primary factor affecting fat digestion in young broilers. In vitro digestion studies confirmed that lipase activity, rather than lipase quantity, primarily influences lipid digestion in young broilers (Michels et al., 2022). Research shows that variations in lipase activity significantly impact lipid digestion kinetics, emphasizing the

importance of the interaction between BAs concentration and lipase activity in achieving an optimal enzyme activity over production phases. For example, Michels et al. (2022) indicated that lipid digestion was inhibited by higher bile salt concentrations at critically low lipase activities.

Brenes et al. (2008) evaluated lipase and/or phospholipase (0.1% diet) supplementation in male broiler chicks (Cobb-500 genetic line) fed diets with 25% high oleic acid sunflower seeds from 4-21 days old. Feeding sunflower seeds negatively affected chickens' performance, fat utilization, plasma cholesterol, pancreatic enzyme activity, and digestive organ morphology but the supplementation of lipase and phospholipase alleviated these effects and resulted in a performance similar to chickens that received the control diet.

Nagargoje et al. (2016) evaluated the effects of soy lecithin, with and without added lipase, on broiler chickens' growth performance, carcass traits, meat quality, blood parameters, and economic efficiency over a 42-day trial. Broilers received a standard diet supplemented with 2%–3% soy lecithin with and without lipase at 150 mg/kg feed in addition to a control group. Lecithin + lipase supplementation greatly increased BWG and improved FCR than the control and lecithin groups, indicating enhanced fat digestion and feed efficiency due to lipase supplementation. Also, the muscle-to-bone ratio and carcass yield were higher, and fat content was lower in this group reflecting increased lean meat production and improved fat metabolism. Furthermore, the meat in this group showed better preservation quality and a longer shelf life, indicating enhanced resistance to spoilage during storage. Additionally, lecithin + lipase supplementation increased the hemoglobin levels and RBC count while the WBC counts remained within normal limits, thus suggesting improved oxygen transport with no adverse immune effects. Finally, lecithin + lipase supplementation significantly reduced cholesterol and TG levels indicating enhanced lipid metabolism and decreased fat accumulation in the blood.

Wang et al. (2018) evaluated the impact of supplementing lipase (4 or 6 U/g) from YL fungus on growth performance over 42 days. Lipase supplementation significantly improved FCR and had no adverse health effects compared to the control group.

Hu et al. (2018) evaluated the effect of lipase supplementation (0.15-0.3 g/kg% diet) on broilers over 28 days. Lipase supplementation significantly increased BWG and improved FCR, fat digestibility, villus height and lipase activity. Also, lipase supplementation decreased the relative weight of abdominal fat and increased the relative weight of the bursa of Fabricius. Additionally, it reduced serum TC, TG, and LDL levels, while HDL levels remained unaffected, suggesting an improved absorption and metabolism of dietary fats.

Studies investigating the effects of exogenous lipase supplementation on the gut morphology of broilers are limited. However, Hu et al. (2018) found that supplementing 0.3% lipase significantly increased villus height and the villus height to crypt depth ratio. These results suggest that lipase supplementation may enhance the digestive and absorptive capacity of the small intestine, likely through an improved nutrient flow, which can support optimal growth in broilers. Similarly, villus height was greater in broiler chickens fed a low-energy diet (reduced 150 kcal/kg of ME) supplemented with lysophospholipid plus lipase than those fed lipase alone. However, crypt depth was greater with lipase supplementation to low energy diet compared to broilers fed standard energy diets but did not differ from those fed low energy diet without supplementation (Movagharnejad et al., 2020).

Oliveira et al. (2019) supplemented lipase (10000 U/kg feed) with or without an emulsifier (250 g/ton feed) to broilers fed diets with a 90-kcal reduction in ME over 37 days. Lipase supplementation alone restored the reduction in BWG caused by the decrease in ME, however, it did not improve FCR, fat digestibility, and carcass yield. Similarly, Wickramasuriya et al. (2020) indicated that adding 0.05% lipase with 0.05% emulsifier to broiler chickens fed diets with 100 kcal reduction in ME over 28 days did not affect BWG, FCR, relative organ weight, serum lipid metabolites, gut morphology and immune response, however, it increased liver weight compared to the emulsifier treatment.

Adding 0.04% lipase with lysolecithin to broilers fed low-energy diets over 35 days was associated with higher BWG and FI but did not affect FCR, nutrient digestibility, serum lipid metabolites, liver enzyme activity and breast muscle fatty acid composition (Ahmad

et al., 2023). In a similar study, adding lipase (0.05 g/kg diet) with lecithin increased BWG and improved FCR and European production index but did not affect FI and carcass traits (Shabani et al., 2021). Interestingly, lipase plus lecithin increased the antibody titers against Newcastle disease and infectious bronchitis, highlighting an immunostimulant role of lipase. However, the relative weight of the thymus was decreased with lipase plus lecithin supplementation.

Nevertheless, research indicates that feeding exogenous lipase alone lacks consistent effects on growth performance, feed efficiency and fat metabolism in broilers (Fernandes et al., 2023). Moreover, studies on co-administration of lipase and BAs in broilers fed standard diets are rare. Thus, one of this study's aims is to explore the synergetic effect of lipase plus BAs on broiler chickens' performance and health.

CHAPTER III

MATERIALS AND METHODS

3.1 Material

The study protocol was approved by the ethics committee of Mersin University for animal experiments: Approval Number: E-37020340-325.04.03-13081607, date: 02.02.2024.

3.1.1 Farm Location

This research was conducted on Güntaş Tavukçuluk farm located at Hamzabeyli Mahallesi Hamzabeyli sokak no: 80 Toroslar Mersin. The farm is located to the northeast of the center of Mersin city, in a mountainous area, 32 km away from the seashore. The farm consists of two barns with a total capacity of 80,000 birds. It operates on a closed-rearing system where temperature and cooling are controlled by a precise electronic system.

3.1.2 Housing and Management

Before the arrival of chickens, the farm was cleaned, washed, and fumigated using ALFUCID which contains 2-PHENYLPHENOL 92% as active material (Figure 3.1).



Figure 3. 1. Housing management. A. fumigation of the poultry house, B. A commercial product that has been used in the fumigation process containing phenyl as an active substance, C. the poultry house after it had been fumigated and disinfected

Feeders and drinkers were disinfected using a disinfectant VIRKON S, which contains potassium peroxymonosulfate 21.4%. The poultry house was provided with thermostats and electrical heaters to provide an appropriate temperature for each stage of growth, and the temperature was automatically controlled at 30-33, 28-29, 25-27, 23-24, and 21-22 °C at the first, second, third, fourth, and fifth weeks, respectively (Figure 3.2).

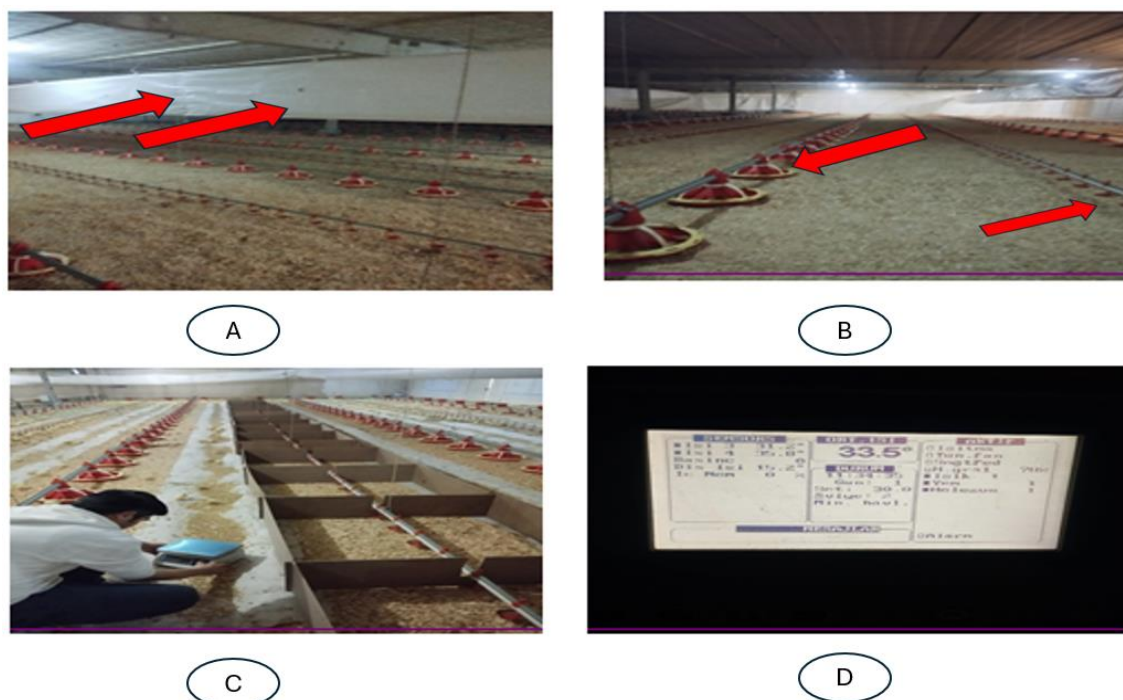


Figure 3. 2. Housing equipment. A. heating system, B. lighting, feeding, and drinking system, C. weighing and distribution of chickens to respective treatment groups, D. control of temperature and ventilation in each part of the poultry house

The farm was well-lit and well-ventilated, providing optimal conditions. so the birds received continuous lighting at an intensity of $16 \text{ lux} / \text{m}^2$ during the first two weeks of age. after the first two weeks they were provided with two hours of darkness and 22 hours of lighting daily until the end of the rearing period maintaining the same light intensity. During the first week, all the feeders and drinkers were adjusted to the appropriate height for the chickens, ensuring they had continuous access to fresh, clean water around the clock, every day of the week.

3.1.3 Chickens

To conduct this research, 240 one-day-old Ross 308 broiler chickens were taken from a commercial company, the birds were assigned to four treatments with four replicates of 15 birds each (mixed males and females), and all the birds were weighed on the first day using an electrical balance with a precision of 0.01 g (Figure 3.3).



Figure 3. 3. Weighting of the birds. A. weighting the birds on the first day, B. assigning chickens to their respective treatment group, C. feeders and waterers for chickens in each treatment replicate

3.1.4 Diets

Commercial broiler feeds (Table 3.1), obtained from a commercial company (Şenpiliç), were fed to Ross 308 according to their age and nutrient requirements (Table 3.2) according to NRC (1994) recommendations. Three diets were fed: starter for the first two weeks, grower for the next two weeks, and the finisher for the final week. All the birds had free access to water and feed.

Table 3. 1. Ingredients composition of basal diets

Ingredients %	Starter (1-14 D)	Grower (15-28 D)	Finisher (29-35 D)
Yellow corn	58	63.27	66.7
Bone owns	2.50	2.10	2
Soybean meal, 46% CP	34.77	30.5	26.2

Table 3. 1. (Devami) Ingredients composition of basal diets

NaCl	0.38	0.38	0.35
Soya oil	0.60	1	2
Calcium carbonate	1.50	1	1
Sodium bicarbonate	0.10	0.1	0.1
MCP	1.40	1	1
DL-Methionine	0.35	0.2	0.2
L-Lysine	0.20	0.3	0.3
L-Threonine	0.10	0.05	0.05
Broiler premix	0.10	0.1	0.1
Total	100	100	100
Premix Analysis in 1 kg			
	Starter	Grower	Finisher
Vitamin A IU/ KG	11.700	9.900	7.500
Vitamin D IU/ KG	4.500	4.050	3000
Vitamin E MG/KG	12.000	12.000	12.000
IRON (IRON SULFATE) MG/KG	20	20	20
COPPER (COPPER SULFATE) MG/KG	16	16	16
ZINC (ZINC SULFATE) MG/KG	120	120	120
Iodine (CALCIUM IODATE) MG/KG	1.25	1.25	1.25

Table 3. 1. (Devamı) Ingredients composition of basal diets

Manganese(manganese oxide) MG/KG	120	120	120
Selenium (Sodium selenate) MG/KG	0.30	0.30	0.30
Phytase enzyme (FTU)	1500	1500	1500
XYLANASE (VU)	1250	1250	1250
GLUCANASE (VU)	860	860	860
Coccidiostat (ppm)	50	50	69

Table 3. 2. Nutrient composition of basal diets (calculated values)

	Starter (1-14 D)	Grower (15-28 D)	Finisher (29-35 D)
ME, Kcal/kg	2950	3100	3250
Crude protein, %	22.43	21.07	18.70
Ether extract, %	4.50	5.22	6.03
Ash, %	5.25	4.40	3.67
Fiber, %	2.88	3.31	3.71
Ca, %	0.96	0.84	0.80
Available Phosphorus %	0.53	0.46	0.41
Lys %	1.4	1.26	1.06
Met %	0.45	0.38	0.52
Met+cys %	1.08	1.07	1.09
Thr %	0.13	0.12	0.14

3.1.5 Experimental Design

Chickens were randomly assigned into four treatment groups including 1) a control group, receiving basal diet only; 2) an antibiotic group: received basal diet plus oxytetracycline hydrochloride 99.8% (OTC) as growth promotor at a dose of 100 mg/kg feed (Veyongpharma company, China); 3) BAs group: received basal diet plus BAs at a dose of 0.27 g/kg feed in the first period (weeks 1 and 2) and 0.25 g/kg feed in the second period (weeks 3-5); and 4) Lipase-BAs group (BAs-L): received basal diet plus BAs as mentioned in BAs group plus lipase at 0.18 g/kg feed (YEMZIM LPZ, ORBA Biokimya San. ve Tic. A.Ş) (Table 3.3).

OTC was only used for experimental purposes and the tested broilers were destroyed after completing the study. BAs were provided by the Lachance company, China under the commercial name RONEON 30% and composed of 297 g of DCA, 66 g of CDCA, and a corn starch (non-active ingredient or carrier) up to 1 kg (The main components of Roneon are BAs extracted from poultry gallbladder bile).

Table 3. 3. Certificate of lipase specifications (ORBA Biokimya San. ve Tic. A.Ş)

Products Name	YEMZIM LPZ Fungial lipaz
Unit Per kg	Min 2 125 000 U/k
Appearance	Whitish powder
Standardized Activity	Lipase (pH 7,5/40oC)
Carrier	Calcium carbonate
Increases Metabolic Energy %	10%
Thermostability: at 95 c and Saturated Moisture for Time 5 min Remaining Activity:	> 90%
Microbiological Properties: Total Viable Count	< 5 * 10 ⁴ cfu/g

BAs, OTC, and lipase were first mixed with an appropriate amount of the basal diet and then added to the respective treatment diet. Birds were individually weighted at 1,7,14, 21, 28, and 35 days old, and FI was calculated weekly until the end of the experiment's period on a replicate basis (Figure 3.4).



Figure 3. 4. Addition of bile acid and lipase to broilers diets. A. bile acids, B. preparation and mixing of the diets, C. lipase enzyme, D. bile acids, E. antibiotic growth promoter (OTC)

Birds were vaccinated, via drinking water, with Bio Vac LS H120 (infectious bronchitis), GUMBORO D78 (Nobilis) and Bio Vac CLONE at 6, 12, and 21 days old, respectively (Figure 3.5 a, b, and c, respectively).

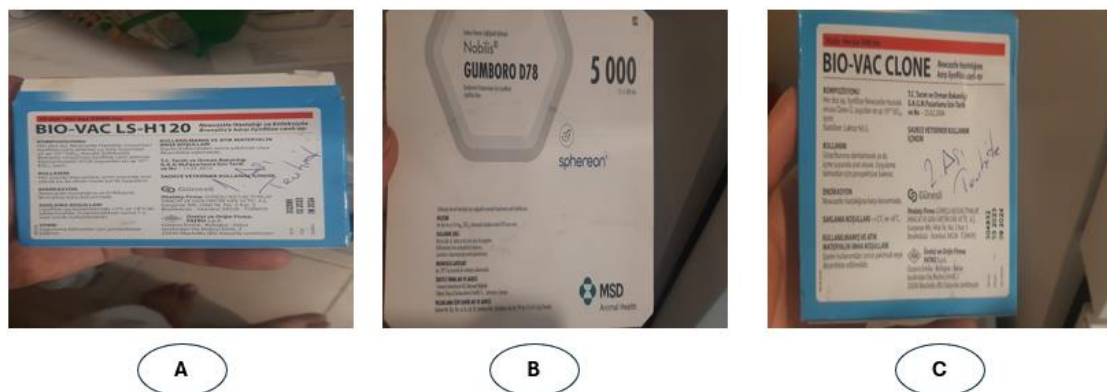


Figure 3. 5. Vaccines administered to broilers during the experiment

3.2 Parameters Measured

3.2.1 Growth Performance

All the birds were weighed on the first day of the experiment by using a digital balance with an accuracy of 1 g then the birds were randomly assigned to the four treatment groups considering that the means of the total weight in each replicate was almost matched. The change in body weight gain was calculated using the following formula:

Body Weight Gain (g) = Final Weight (g) – Initial Weight (g) (Figure 3.6)



Figure 3. 4. Weighing birds at the end of each week during the experimental period

The feed was provided ad libitum throughout the experiment, allowing the birds to consume food as needed. At the end of each week, the refused feed was gathered and weighed, and the offered feed was weighed weekly for each replicate in all treatments. By subtracting the amount of refused feed from the amount of offered feed, the FI for each replicate was calculated and as a result for each replicate, the quantity of feed

consumed was divided by the total number of birds to calculate the weekly individual FI amount.

The following method was used to obtain the weekly FCR:

$$\text{FCR} = \text{Feed Consumed (g)} / \text{BWG (g)}$$

Protein efficiency ratio was calculated by dividing BWG (g) by protein intake (g) (Kamran et al., 2008). The energy efficiency ratio was calculated by dividing BWG (g) by the energy consumed (kcal) and multiplying the result by 100 (Kamran et al., 2008).

European production efficiency factor was calculated by multiplying livability and live weight (kg) by 100 and dividing the result by FCR and age (days)

$$\text{EPEF} = (\text{Livability} * \text{Live Weight (KG)} * 100) / (\text{FCR} * \text{Age (day)}) \text{ (Shoaib et al., 2021).}$$

3.2.2 Carcass Characteristics, Relative Organ Weights and Meat Quality

At the end of the experiment, the birds starved for 10 hours and subsequently, 12 birds from each treatment (3 birds per replicate) were slaughtered, the feathers, head, neck, and legs were removed, all internal organs were collected, and their relative weights were measured (Figures 3.7-3.9). The weights of the carcass's major parts (thighs, breast) were weighed using a scale with a precision of 0.001g (Necklife, Türkiye) to calculate the ratios of the carcass's major components to the total carcass weight. So, the relative organ weight (fraction) to the carcass weight of the birds was calculated in percentage (Ismael et al., 2022), and only the small intestine weight was calculated in a percentage of live body weight (Alzawqari et al., 2016).

$$\text{Fraction Weight, \%} = (\text{Fraction Weight (g)} / \text{Carcass Weight (g)}) \times 100$$

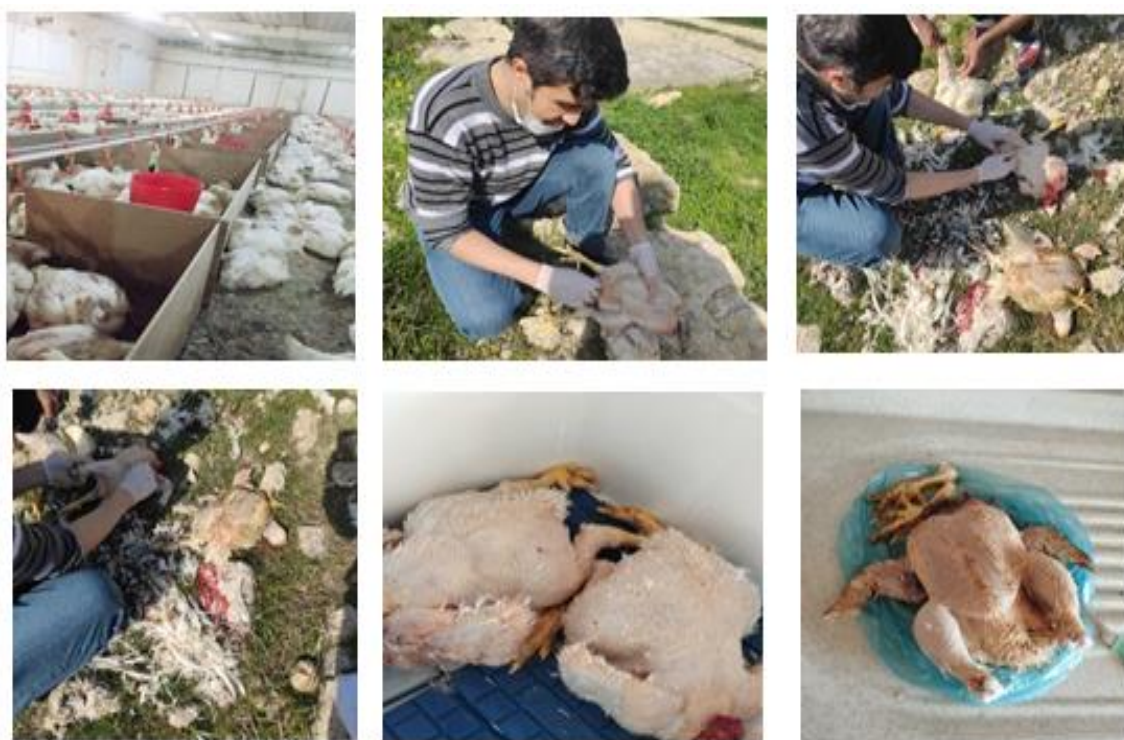


Figure 3. 5. Slaughtering the birds after feed withdrawal for 10 hours



Figure 3. 6. Scaling of slaughtered chickens

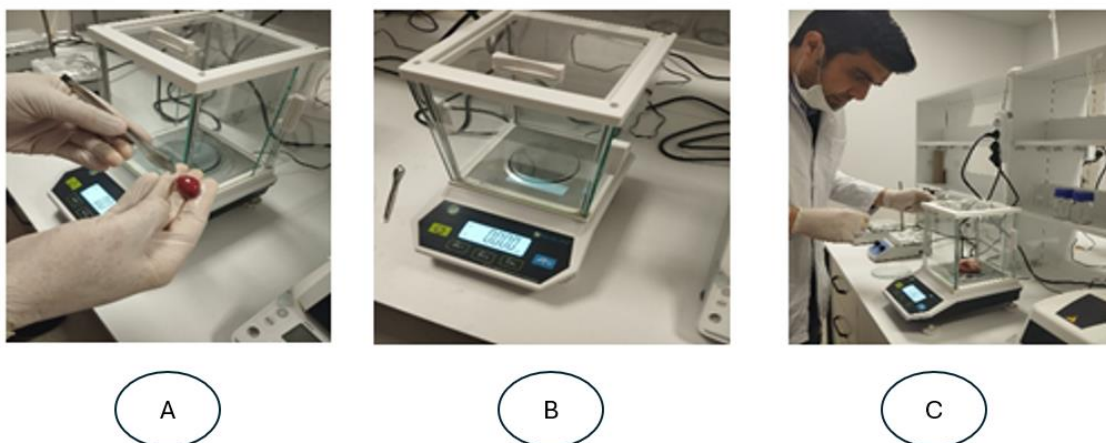


Figure 3. 7. A and C. weighing of small internal organs, B. high accuracy scale with precision of 0.001g

The dressing percentage was determined by dividing the carcass weight including giblets weight (liver, heart and gizzard) by the live weight multiplied by 100 (Ismael et al., 2022).

$$\text{Dressing Percentage} = (\text{Carcass Weight (With Giblets)} / \text{Live Weight}) * 100$$

To determine the pH value of breast meat, approximately 5 g of breast meat was taken and mixed into 10 ml of distilled water and the pH value in breast muscles was measured using a pH meter (benchtop-sper scientific) (Figure 3.10).



Figure 3. 8. A. pH meter apparatus, B. homogenization of the meat sample with distal water, C. the meat sample after being homogenized, D. measuring the pH value of the sample

For the determination of meat cooking loss, approximately 40 g of breast muscle was taken and cooked at an internal temperature of 75 °C in a water bath for 30 minutes. Then, the meat was put on a dry surface for 10 minutes. After that, it was reweighed, and the cooking losses were calculated. The measurement was repeated 3 times for each replicate of each treatment group (Figure 3.11).

Cooking Losses (G) = Fresh Meat Weight (G) – Cooking Meat Weight (G) (Shoaib et al., 2021).



Figure 3. 9. Preparing the water bath and meat samples

The moisture content in meat was determined using a moisture meter apparatus (Shimadzu model moc63 u). A one g of breast meat was taken, and the moisture of each sample was recorded. The measurement was repeated 3 times for each replicate of each treatment group (Figure 3.12).



Figure 3. 10. Moisture meter apparatus

3.2.3 Blood Parameters

Blood samples were obtained on the final day of the experiment (d 35) from 12 birds in each treatment (3 birds from each replicate) from the wing vein. Each blood sample was divided into two portions, one with EDTA to evaluate the white blood cell count (WBC) only. The second portion was without EDTA and used for measuring the serum content of lipid metabolites (TG, HDL, LDL, and cholesterol), immune metrics (total protein, albumin and IgG), liver function indices (ALT, AST and bilirubin), kidney function indices (uric acid, creatinine) and digestive enzyme activity (amylase and lipase). All samples were centrifuged at 3000 RMP for 10 minutes and stored at -20 c until analysis. Laboratory analysis was performed at DOLLVET BIOTECHNOLOGY at Şanlıurfa using a spectrophotometer (Thermo Scientific model 300) with respective kits (Figure 3.13).



Figure 3. 11. Blood sampling and processing. A. blood obtained from wing vein, B. blood samples, C. blood tube with EDTA, D. blood tube without EDTA, E. spectrophotometer, F. Centrifuge

The cholesterol, HDL, LDL, and TG were measured using specific kits (RANDOX POULTRY LIPID KIT, LPD CONTRO 3) at wavelengths of 490 nm for cholesterol, 600

nm for HDL and LDL, and 500 nm for TG. The serum total protein was measured using a total protein kit (Sigma-Aldrich, Biuret method) at a wavelength of 540 nm while albumin was measured at a wavelength of 630 nm using BCG Albumin assay kit BCG (Sigma-Aldrich). In addition, the IgG levels were measured using a chicken IgG Elisa kit (MyBioSource) at a wavelength of 450 nm (Figure 3.14).



Figure 3. 12. A. Randox poultry lipid, B. total protein kit (Sigma-Aldrich, Biuret method), C. chicken IgG Elisa kit (MyBioSource)

The liver function enzymes ALT and AST were measured using ALT/GPT reagent (Randox kit) and AST/ GOT reagent (Randox kit), respectively with a wavelength of 340 nm. The total bilirubin and direct bilirubin were assayed using a bilirubin reagent (Randox kit) with a wavelength of 540 nm. The Creatinine Assay Kit (ELISA Technologies, Inc.) was used to measure creatinine at a wavelength of 546 nm. A Uric Acid Test Kit (Sigma-Aldrich) was used to measure uric acid at a wavelength of 340 nm. For digestive enzymes amylase and lipase assay, an Amylase Assay Kit (BioVision Technologies) and lipase Activity Assay Kit (BioVision Technologies) with wavelengths of 405 nm and 540 nm were used to measure amylase and lipase, respectively.

3.2.4 Cecal total bacterial counts

At the end of the experiment and after the birds were slaughtered, the caecal fresh contents from 12 birds of each treatment group (3 birds per replicate) were collected into special tubes. The tubes were preserved in containers containing ice made from carbon dioxide and fitted with a datalogger to monitor the temperature, ensuring that the samples

remained unaffected by temperature fluctuations during transport, to avoid adverse impact on bacteria counts (Alagawany et al., 2018; Mohamed et al., 2020) (Figures 3.15 and 3.16).



Figure 3. 13. Transporting of cecal contents. A. caecal fresh contents, B. dry ice, C. datalogger apparatus

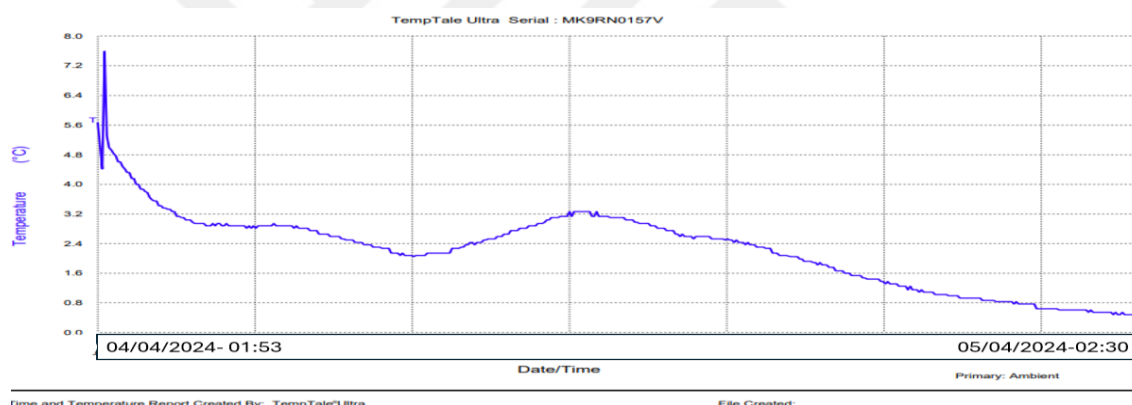


Figure 3. 14. Datalogger information

For determining the cecal total bacterial counts (TBCs), one gram of caecal contents was homogenized in 9 mL of 0.1% peptone water for one minute. This homogenate was then diluted with 0.1% peptone water and Ringer's solution to create serial dilutions of the caecal contents, as illustrated in Figure 17.

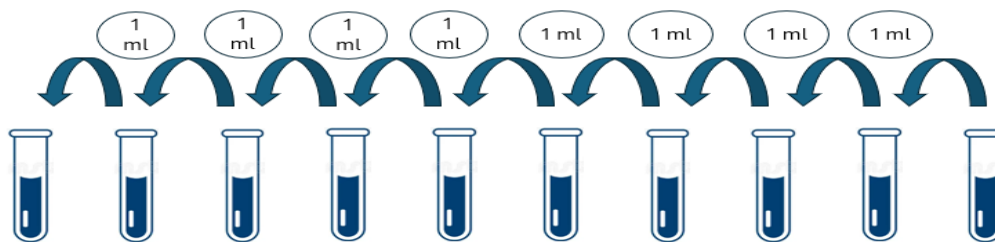


Figure 3. 15. Making serial dilutions of caecal contents

The plate count agar (PCA) medium was prepared by dissolving 23.5 g of PCA powder in 1 liter of distilled water and was autoclaved for 15 minutes at 121°C for sterilization. The medium was then added to 12.5 mL Petri plates. The Spread Plate Method was employed to determine the total bacterial count. Briefly, 0.1 mL (100 microliters) of the diluted bacterial sample was carefully spread over the surface of the agar plate using a sterile spreader (Figures 3.18 and 3.19).

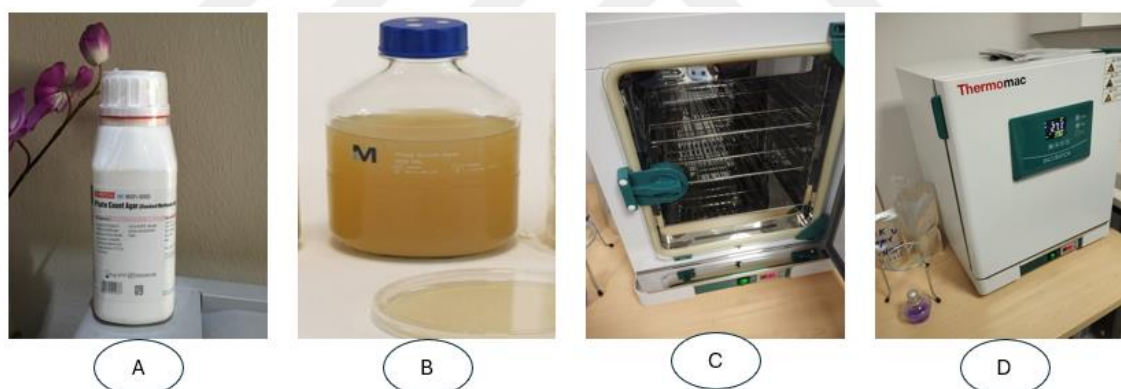


Figure 3. 16. Processing of cecal content and preparing the media for bacterial count.
A and B. plate count agar, C and D. Incubator

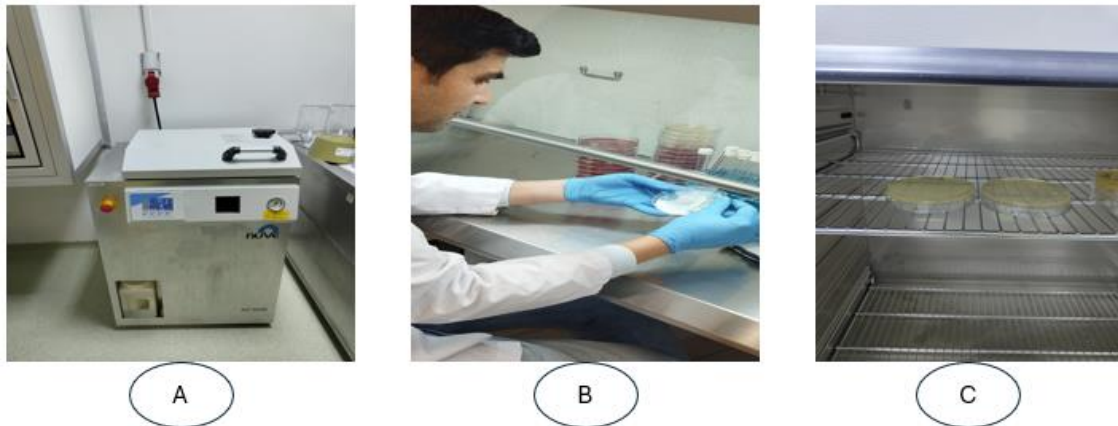


Figure 3. 17. Counting total bacteria in cecal contents. A. sterilizing in an autoclave for 15 minutes at 121°C, B. spread plate method, C. petri plates in the incubator

After two days of incubation at 30°C, there were three kinds of Petri plates:

The TNTC (too numerous to count) and TFTC (too few to count) were neglected while plates with a colony count between 30 and 300 colonies were considered acceptable. The bacterial count was expressed as Log CFU/g, which represents the logarithm of the colony-forming units per gram of the sample (Figure 3.20). The determination of cecal TBCs was performed at DOLLVET BIOTECHNOLOGY Şanlıurfa.

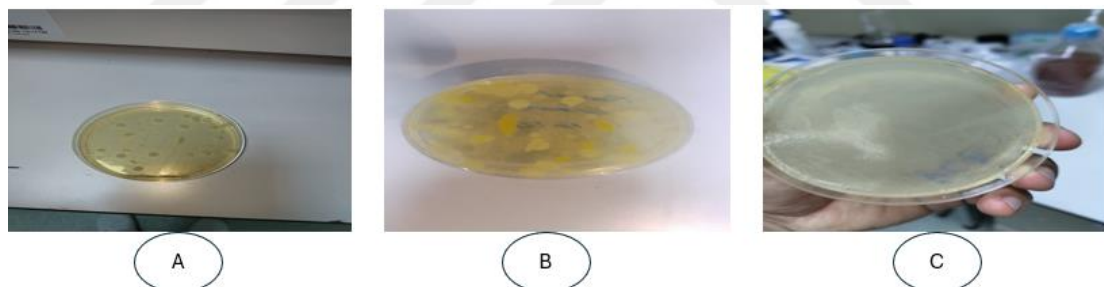


Figure 3. 18. Counting the colonies of bacteria. A. accepted plate, B. TNTC plates (neglected), C. TFTC plates (neglected)

3.3 Statistical Analysis

The One-Way ANOVA of SPSS (version 18.0, SPSS Inc., Chicago, USA) was used to evaluate the data. The Duncan Multiple Range Test was applied to determine the significance of differences among treatment means. A significance level of $P < 0.05$ was used. Data are presented as means $\pm$ SEM.

CHAPTER IV

RESULTS AND DISCUSSION

Supplementation of lipase and BAs in broiler diets has gained attention for its potential impact on growth performance and health. While studies indicate the limited efficacy of lipase alone, its function can be significantly improved when combined with BAs. Notably, there is no research directly comparing BAs as an alternative to AGPs like OTC in broilers. Therefore, this study investigated the synergistic effect of dietary BAs and lipase co-supplementation on growth performance, metabolic profile, immunity, carcass characteristics, and meat quality. It further explored the potential of BAs as an alternative to AGPs like OTC. This research is essential for understanding whether BAs can effectively replace AGPs while improving overall broiler health and performance.

4.1 Growth Performance and Feed Efficiency

The weekly changes in body weight development over the 5-week experiment are illustrated in Table (4.1). Broilers supplemented with BAs showed the highest body weight in the 2nd week ($P < 0.05$) while the broilers fed both lipase and BAs showed the highest body weight at the 5th week ($P < 0.05$), compared to OTC and control groups. There was no significant difference between BAs and BAs plus lipase or between OTC and control treatments at all experiment periods (Table 4.1).

Table 4. 1. Body weight development of broilers supplemented with BAs, lipase and OTC

Treatment	Period					
	Day 1	1st Week	2 nd Week	3 rd Week	4 th Week	5 th Week
Control	A 40.12	A 215.0	B 530.05	A 1004.0	A 1585.0	C 2059.98
OTC	A 40.12	A 225.0	AB 532.03	A 1007.0	A 1590.0	B 2073.15
BAs	A 40.22	A 216.19	A 541.05	A 1011.0	A 1598.0	A 2156.11
BAs-L	A 40.33	A 226.04	AB 538.02	A 1013.0	A 1596.0	A 2166.27
SEM	0.122	0.166	2.78	0.125	0.129	2.74
P-value	0.67	0.65	0.045	0.55	0.56	0.041

- Means within the same column with different letters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

The increase in weekly BWG and total BWG over the 5-week experiment are shown in Table (4.2). During the first week of age, results indicated that higher BWG was observed in both lipase plus BAs and OTC groups compared to both control and BAs groups ($P < 0.05$). In the 5th week and the whole cumulative period, the increase in BWG was recorded for the lipase plus BAs group followed by the BAs group compared to both OTC and control groups ($P < 0.05$). There was no significant difference between lipase plus BAs and BAs groups or between OTC and control groups during the 5th week and the whole cumulative period (Table 4.2).

Table 4. 2. Body weight gain development supplemented with BAs, lipase and OTC

Treatment	Period					
	1st Week	2 nd Week	3 rd Week	4 th Week	5 th Week	Total Body Gain (1-35 g)
Control	B 174.88	A 315.05	A 473.95	A 581.00	B 474.98	B 2019.86
OTC	A 184.88	A 307.03	A 474.97	A 583.00	B 483.15	B 2033.03
BAs	B 175.97	A 324.86	A 469.95	A 587.00	A 558.11	A 2115.89
BAs-L	A 185.71	A 311.98	A 474.98	A 583.00	A 570.27	A 2125.94
SEM	1.14	0.170	0.178	0.129	2.14	2.88
P-value	0.047	0.66	0.45	0.56	0.014	0.03

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

The changes in weekly FI for different treatments are presented in Table (4.3). Dietary supplementation of lipase plus BAs and BAs decreased the FI during the 5th week and the whole period compared to the OTC and control groups ($P < 0.05$). The higher FI was observed in the control group followed by the OTC group (Table 4.3).

Table 4. 3. Feed intake of broilers supplemented with BAs, lipase and OTC

Treatment	Period					
	1st Week	2nd Week	3rd Week	4th Week	5th Week	Cumulative Feed Intake (1-35 d)
Control	A 210.0	A 378.0	A 570.0	A 977.0	A 1040.0	A 3175.0
OTC	A 205.0	A 375.0	A 565.0	A 970.0	A 1033.0	B 3148.0
Bas	A 190.0	A 370.0	A 563.0	A 930.0	C 998.0	C 3051.0
BAs-L	A 193.0	A 371.0	A 562.0	A 927.0	B 1000.0	C 3053.0
SEM	0.155	0.172	0.179	0.130	2.17	2.98
P-value	0.47	0.65	0.55	0.57	0.014	0.02

- Means within the same column with different letters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

Table (4.4) shows the effect of various treatments on the FCR of broilers. There was a significant difference in FCR among treatments in all weeks except in week 3. In the first week, broilers fed BAs plus lipase showed enhanced FCR followed by those fed BAs whereas, in week 2, broilers fed BAs showed an improved FCR followed by those fed lipase plus BAs, compared to control and OTC treatments ($P < 0.05$). for the 4th and 5th weeks as well as the whole cumulative experimental period, the enhanced FCR was

observed in broilers supplemented with BAs and BAs plus lipase compared to both control and OTC groups ($P < 0.05$) (Table 4.4).

Table 4. 4. Feed conversion ratio in broilers supplemented with BAs, lipase and OTC

Treatment	Period					
	1st Week	2 nd Week	3 rd Week	4 th Week	5 th Week	Cumulative FCR (1-35 d)
Control	A 1.20	A 1.20	A 1.20	A 1.68	A 2.19	A 1.57
OTC	B 1.11	A 1.22	A 1.19	A 1.66	A 2.14	A 1.55
BAs	B 1.08	B 1.14	A 1.20	B 1.58	B 1.79	B 1.44
BAs-L	C 1.04	A 1.19	A 1.18	B 1.59	B 1.75	B 1.44
SEM	2.55	1.18	0.16	2.15	2.19	2.99
P-value	0.042	0.041	0.51	0.023	0.014	0.03

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

Table (4.5) shows the effects of treatments on the efficiency of protein utilization (PER) in broilers. The weekly and cumulative value of PER differs significantly ($P < 0.05$) among treatments and was higher in the BAs and lipase plus BAs groups than in the OTC and control groups. There were no significant differences in PER between OTC and

control groups. The PER was higher for BAs alone compared to BAs with lipase supplementation at weeks 2 and 4, but lower at week 3.

Table 4. 5. PER of broilers supplemented with BAs, lipase and OTC

Treatment	Period					
	1st Week	2 nd Week	3 rd Week	4 th Week	5 th Week	Total PER (1-35 d)
Control	C 3.71	B 3.71	C 3.94	B 2.82	B 2.44	B 3.32
OTC	B 4.02	B 3.64	C 3.99	B 2.85	B 2.50	B 3.40
BAs	A 4.12	A 3.91	B 3.96	A 2.99	A 2.99	A 3.59
BAs-L	A 4.28	B 3.74	A 4.01	B 2.98	A 3.05	A 3.61
SEM	2.56	2.17	2.18	2.19	2.22	2.95
P-value	0.031	0.03	0.03	0.013	0.02	0.04

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. PER: protein efficiency ratio. SEM: standard error of means

Table (4.6) presents the response of energy efficiency utilization (EER) to various treatments in broilers. Except in the 3rd week, the weekly and cumulative EER differ significantly ($P < 0.05$) among treatments. Compared to both OTC and control groups, the highest EER values were recorded for both BAs and BAs plus lipase treatments without significant differences among them except in the 2nd week where the BAs group was higher than the BAs plus lipase group. The EER was similar between the control and

OTC treatments except for the 1st and 2nd weeks, where the OTC group exhibited higher and lower values, respectively, compared to the control group.

Table 4. 6. EER % of broilers supplemented with BAS, lipase and OTC

Treatment	Period					
	1st Week	2nd Week	3rd Week	4th Week	5th Week	Total EER (1-35 d)
Control	B 28.22	AB 28.26	A 26.83	B 19.18	B 14.73	B 23.44
OTC	AB 30.57	B 27.75	A 27.12	B 19.39	B 15.08	B 23.98
BAs	A 31.36	A 29.78	A 26.93	A 20.36	A 18.04	A 25.29
BAs-L	A 32.61	AB 28.51	A 27.26	A 20.30	A 18.39	A 25.41
SEM	2.58	2.19	2.19	2.18	2.25	2.98
P-value	0.022	0.02	0.04	0.012	0.03	0.04

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. EER: energy efficiency ratio. SEM: standard error of means

Table (4.7) illustrates the value of EPEF in response to various treatments in broilers. EPEF, a comprehensive measure of production performance, was significantly increased in the BAs and BAs plus lipase groups compared to the control and OTC groups ($P < 0.05$). The BAs group showed the highest value followed by the BAs plus lipase, OTC, and control group, respectively.

Table 4. 7. EPEF in broilers supplemented with BAs, lipase and OTC

Treatment	Total EPEF
Control	C 374.42
OTC	C 382.50
Bas	A 420.09
BAs-L	B 402.36
SEM	3.29
P-value	0.012

- Means within the same column with different litters are significantly different at ($P < 0.05$).
- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. EPEF: European production efficiency factor. SEM: standard error of means

Thus, this study demonstrates that dietary supplementation with BAs, either alone or in combination with lipase, significantly enhanced BWG, reduced FI and improved FCR. These beneficial effects were more pronounced with the co-supplementation of BAs and lipase. This improvement can be attributed to the enhanced digestion and absorption of nutrients facilitated by BAs and lipase. Our findings agree with those concluded by Alzawqari et al. (2016) and Lai et al. (2018) who stated that there were significant improvements in the BWG of chicks supplemented with BAs. Also, Brzóška et al. (2013) have shown that broiler growth improved with supplementing 0.3–0.9% BAs. Enhanced lipid digestion through BAs increases the bioavailability of fat-soluble vitamins (A, D, E, and K) which consequently contributes to improved BWG (Yang et al., 2022).

Decreasing FI with dietary BAs supplementation is in harmony with the findings of Alzawqari et al. (2016) who observed that broilers supplemented with 0.25 and 0.50% DBA had higher body weight, body weight BWG, and lower FI than the non-

supplemented ones. Conversely, Parsaie et al. (2007) reported a significant increase in both FI and BWG with no change in FCR in broilers supplemented with BAs compared to the control treatment. Similarly, Lai et al. (2018a) reported improved BWG and FCR without affecting FI in broilers fed BAs. Also, Karimi et al. (2011) reported that the inclusion of BAs in broiler feeds did not improve FI. In the study by Piekarski et al. (2016), CDCA supplementation in broilers upregulated appetite-related hypothalamic neuropeptides, leading to a decrease in FI. However, this reduction in FI was accompanied by a decrease in BWG.

The results of PER, EER % and EPEF indicated better utilization of dietary protein for growth in the BAs and lipase-supplemented groups. This aligns with the known role of BAs in facilitating protein digestion and absorption. Gass et al. (2007) demonstrated in vitro that BAs enhance the proteolytic activity of pancreatic enzymes, including trypsin and chymotrypsin. Also, higher protein digestibility was reported in broilers fed diets supplemented with BAs (Shoaib et al., 2022). Conversely, protease activity was linearly declined in broilers fed increasing levels of BAs compared to the control (Mohamed et al., 2020). This controversy might be attributed to variations in the source, composition and doses of BAs used in different studies, in addition to varying dietary composition and the ages of the chickens. Generally, it has been proposed that the response of digestive enzyme activities to dietary changes varies with the age of birds (Cristiane et al., 2014; Liu and Kim, 2017). Also, BAs plus lipase supplementation enhanced energy efficiency in our study, possibly by improving lipid digestion and utilization. This indicates that the overall production efficiency of broilers was greatly improved with BAs plus lipase supplementation in our study, as previously proposed (Maisonnier et al., 2003). These findings are consistent with those of Ganna et al. (2022) who concluded that supplementing broiler diets with BAs improved body weight, BWG, and FCR, while also improving both economic and European production efficiency.

The differences in the impact of BAs across various studies can be attributed to several factors, including chicken breed, diet composition, BAs source, composition and doses, and the rearing systems (Abd El-Aziz et al., 2025; Fernandes et al., 2023; Hanif et al., 2024; Shoaib et al., 2023). For example, different chicken breeds may metabolize BAs differently leading to varied effects on lipid utilization and growth performance. The composition of the diet, particularly the types and amounts of fats, fibers, and other

nutrients included, can significantly affect how effectively BAs facilitate nutrient digestion. Additionally, the specific type and levels of BAs supplemented can produce different results, because some BAs may be more efficient than others in enhancing lipid utilization. Lastly, rearing systems, including conventional versus controlled environments, can affect the gut microbiota, which, in turn, interacts with BAs to influence overall health and performance in broiler chickens. Nevertheless, our results underscore the potential of BAs as an alternative to OTC in terms of growth performance and feed efficiency.

4.2 Carcass Characteristics, Relative Organ Weights and Meat Quality

4.2.1 Carcass Characteristics

Table (4.8) shows the carcass characteristics of broilers fed BAs and/or lipase and the AGP oxytetracycline. The live weight, hot carcass weight, cold carcass weight and dressing percentage differ significantly ($P < 0.05$) among treatments.

The highest values were observed in BAs and BAs-L compared to control and OTC-supplemented groups. The carcass characteristic parameters were similar in OTC and control broilers. Similarly, there was no significant difference between BAs and BAs plus lipase-supplemented chickens.

Table 4. 8. Carcass characteristics of broilers supplemented with BAs, lipase and OTC

Treatment	Item			
	Live Weight (g)	Hot Carcass Weight (g)	Cold Carcass Weight (g)	Dressing Percentage %
Control	B 2059.98	B 1429.51	B 1425.15	B 70.42
OTC	B 2073.15	B 1476.29	B 1471.29	B 71.21
BAs	A 2156.11	A 1624.19	A 1619.19	A 75.33
BAs-L	A 2166.27	A 1629.90	A 1623.90	A 75.24
SEM	2.59	2.70	2.55	1.71
P-value	0.021	0.011	0.012	0.041

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

Thus, BAs supplementation, either alone or in combination with lipase, enhanced carcass characteristics, live weight, and dressing percentage in broilers in this study. These effects are primarily attributed to improved fat and protein digestibility, nutrient absorption, and overall metabolic efficiency, as has been indicated by Shoaib et al. (2022). Feeding emulsifiers and BAs have been shown to reduce abdominal fat and digesta fat (Cheah et al., 2017), as well as increase breast muscle yield (Ge et al., 2019; Geng et al., 2022), thus contributing to improved dressing percentage and carcass quality. Similarly, the dressing percentage and thigh muscle yield as well as duodenal lipase and lipoprotein lipase

activity were increased in broilers fed BAs (Lai, et al., 2018a), further promoting fat and protein digestion and absorption. Although there were no significant differences between BAs alone or in combination with lipase, their co-supplementation exerted numerically higher values for carcass yield compared to BAs alone, probably indicating a synergistic effect of BAs and lipase on nutrient utilization.

In contrast to our results, Arshad et al. (2020) indicated that BAs and lipase supplementation to low-energy diets did not improve carcass yield. Similarly, Karimi et al. (2011) demonstrated that bovine bile powder has no effects on performance, and for better results, this must be associated with a multi-enzyme in broiler diets. It has been proposed in broilers and piglets that the inconsistent effect of exogenous emulsifiers on growth performance can be attributed to the degree of saturation of the dietary fats (An et al., 2020; Soares and Lopez-Bote, 2002) and the unique components in natural BAs (Dierick and Decuyper, 2004; Lai et al., 2018b). These components may act synergistically and produce effects not observed with synthetic or purified BAs. Furthermore, San Tan et al. (2016) reported that supplementation with an exogenous emulsifier based on PGR (Phospholipid-Glycerol-Residue) could be beneficial for chickens fed on diets containing a high unsaturated: saturated fatty acid ratio.

Importantly, the BAs effects were superior to OTC in enhancing carcass yield, highlighting the role of BAs as a potential alternative to APGs. The APGs promote growth primarily by inhibiting harmful bacteria that compete with the host for nutrients. It has been reported that APGs exert their effects by reducing the activity of bile acid hydrolase, an enzyme produced by gut bacteria that negatively affects lipid utilization, and reducing its activity improves feed efficiency and carcass yield. Moreover, APGs have been reported to reduce the activities of beneficial bacteria like lactobacillus which may disrupt intestinal health in the long term (Geng and Lin, 2016; Lin, 2014). Adding OTC (200 mg/kg) to a broiler diet for 35 d did not decrease the weight of abdominal fat relative to the control (Khadem et al., 2014). Thus, the distinct advantages of BAs over APGs in enhancing nutrient utilization, gut health, and absorption of lipid-soluble vitamins position them as valuable alternatives to APGs in poultry production (Geng and Lin, 2016; Knarreborg et al., 2004; Lin, 2014; Yang et al., 2022).

4.2.2 Relative Organ Weights

The relative organ weights are presented in Table (4.9) and the results indicated significant differences ($P < 0.05$) among treatments in various organs except for heart, liver and intestinal weight. Compared to other treatments, the highest breast, thigh, bursa, and spleen relative weights and the lowest abdominal fat relative weights were observed in broilers fed BAs alone or with lipase. Feeding OTC produced the lowest relative weight for thigh muscles and the highest gizzard relative weight compared to other treatments.

Table 4. 9. Relative organ weights in broilers supplemented with BAs, lipase and OTC

Treatment	Item								
	Breast Yield %	Thigh Yield %	Heart %	Gizzard %	Liver %	Abdominal fat %	Bursa %	Spleen %	Small Intestine % of Body Weight*
Control	B 35.51	A 35.65	A 0.804	C 1.121	A 1.893	A 2.41	C 0.149	B 0.178	A 7.910
OTC	B 35.56	B 33.91	A 0.823	A 1.153	A 1.885	A 2.48	B 0.170	B 0.17	A 7.885
Bas	A 37.94	A 35.18	A 0.817	C 1.114	A 1.882	B 1.73	B 0.17	A 0.23	A 7.819

Table 4. 9. (Devamı) Relative organ weights in broilers supplemented with BAs, lipase and OTC

BAs-L	A	A	A	B	A	C	A	A	A
	39.55	35.88	0.807	1.144	1.895	1.58	0.19	0.250	7.928
SEM	3.68	2.22	2.38	2.38	1.25	2.99	3.55	3.59	1.12
P-value	0.021	0.03	0.54	0.013	0.53	0.04	0.05	0.023	0.20

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

This improvement in relative organ weights is attributed to enhanced fat digestion and nutrient absorption and utilization by BAs and lipase, as has been indicated by Shoaib et al. (2022). Feeding BAs has been shown to reduce abdominal fat (Cheah et al., 2017) and increase breast muscle yield (Ge et al., 2019). Similarly, thigh muscle yield was increased in broilers fed BAs (Lai et al., 2018a). Also, breast muscle percentage was greater in broilers fed de-oiled lecithin emulsifier compared to the control (Liu et al., 2020). Recently, it was identified that BAs (250mg/kg diet) increased breast muscle growth by activating the insulin-like growth factor 2 via FXR signaling (Chen et al., 2024).

Our results indicated a significant increase in the relative weight of the bursa of Fabricius and spleen with supplementation of BAs alone or with lipase, suggesting a potential enhancement in immune function since these organs are critical components of the immune system in poultry. The greater IgG levels in our broilers supplemented with BAs and BAs plus lipase further support the immunostimulant role of BAs. Although little information is available on the impact of BAs on systemic immunity, several reports highlighted the benefits of BAs on gut immunity, as has been indicated by enhanced

intestinal defense mechanisms and reduced gut inflammatory response in broilers suffering from necrotic enteritis (Bansal et al., 2020; Bansal, 2021), heat stress (Yin et al., 2021a; Yin et al., 2021b), salmonella infection (Hu et al., 2024a), and endotoxins (Fan et al., 2024).

In contrast, supplementing BAs or lipase to broilers fed an energy-diluted diet did not affect the relative weights of the spleen, bursa, abdominal fat and breast muscle (Arshad et al., 2020). Also, BAs (200g/ton) didn't improve the relative weights of the breast, thigh, gizzard, bursa, spleen, liver, and abdominal fat in broilers (Hussien, et al., 2022). Similarly, feeding de-oiled lecithin emulsifier to broilers did not affect the relative weight of bursa, spleen liver, gizzard and abdominal fat (Liu et al., 2020). A recent study in Geese reported that BAs (up to 300 mg/kg diet) did not affect the weights of the spleen and bursa of the Fabricius (Cao et al., 2022). The discrepancy among different results probably implies that the effectiveness of BAs and lipase may vary depending on varying dietary formulations and conditions, and the composition and dose of emulsifiers, and further research may be needed to clarify these discrepancies.

4.2.3 Meat Quality

Meat quality parameters are presented in Table (4.10). While the pH and moisture % did not significantly change among treatments, the cooking losses were greater for BAs alone or with lipase compared to control and OTC treatments.

Table 4. 10. Meat quality parameters in broilers supplemented with BAs, lipase and OTC

Treatment	Item		
	PH	Moisture %	Cooking Losses, g
Control	A 6.90	A 73.46	B 21.24
OTC	A 6.53	A 73.41	B 21.21
Bas	A 6.07	A 73.84	A 22.48
BAs-L	A 6.45	A 73.56	A 22.79
SEM	1.11	0.93	3.19
P-value	0.22	0.42	0.014

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

Various factors can affect cooking loss, one of them is the water-holding capacity (WHC) of meat which inversely correlates with cooking loss (Mir et al., 2017; Watanabe et al., 2018). The WHC increases with increasing fat bound to protein in meat. Thus, this higher cooking loss may be because BAs enhance fat digestion and absorption by emulsifying fats in the gut. This can reduce intramuscular fat because fat is more efficiently metabolized and stored elsewhere, leaving less fat within the muscle fibers. Intramuscular fat helps retain moisture during cooking, so with reduced fat, water loss during cooking tends to increase i.e. a decrease in WHC. We did not measure WHC, and little research is linking BAs feeding with meat quality, but previous studies reported a decrease in WHC when using dietary emulsifiers (Shoaib et al., 2022; Upadhaya et al., 2018). Also, Hussien et al. (2022) reported a decreased moisture and fat content in the meat of broilers fed BAs (200g/ton, Runeon®). However, in the study of Shoaib et al. (2022), the

cooking loss and pH were unaffected by BAs supplementation despite the greater WHC reported by BAs feeding. In another study, both cooking loss and WHC were increased with feeding an exogenous emulsifier to broilers (An et al., 2020). In contrast, Zhao and Kim (2017) reported no effect of lysophospholipids emulsifier on meat pH and WHC in broilers while Hussien, et al. (2022) reported a reduction in moisture content of meat in broilers fed 200g/ton of BAs (Runeon). Similarly, BAs addition did not affect the meat pH, cooking loss and WHC in broilers fed 200-600 mg/kg BAs from day 8 to day 32 of age (Hasibuan et al., 2025). Changes in fat and moisture balance, fat/protein ratio, or fatty acid composition in meat have been proposed to affect meat quality and may lead to protein denaturation under heat, causing more fluids to be released from the muscle during cooking (Mir et al., 2017; Watanabe et al., 2018). So, the BAs without and with lipase might induce changes in intramuscular fat composition and distribution and modulate the metabolic activity associated with fat utilization, thus contributing to the increased cooking loss in broilers fed BAs-supplemented diets. Further research may be warranted on the effects of BAs and lipase on the fatty acid composition of broiler meat.

4.3 Metabolic Profile

4.3.1 Serum Lipid Profile

Table (4.11) shows the changes in serum lipid profile in response to feeding BAs, lipase or OTC to broilers. Results indicated significant differences ($P < 0.05$) in serum lipid profile parameters among treatments. Broilers supplemented with BAs alone or with lipase showed significantly lower cholesterol, HDL, LDL, and TG levels than broilers fed the control and OTC-supplemented diets. There was no significant difference either between control and OTC diets or between BAs and BAs plus lipase diets, respectively, regarding serum lipid profiles.

Table 4. 11. Serum Lipid profile parameters in broilers supplemented with BAs, lipase and OTC

Treatment	Item			
	CHOLESTEROL mg /dl	HDL mg /dl	LDL mg /dl	Triglyceride mg /dl
Control	A 105.03	A 80.61	A 27.0	A 92.68
OTC	A 106.82	A 80.30	A 24.83	A 92.51
Bas	B 88.42	B 63.70	B 19.66	B 60.79
BAs-L	B 83.07	B 61.79	B 20.10	B 61.48
SEM	2.60	2.40	2.77	2.90
P-value	0.021	0.02	0.03	0.011

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

Serum TG, LDL, and HDL concentrations have been regarded as diagnostic markers in lipid metabolism (Lai et al., 2018b). The synthesis of adipose tissue and fat deposition in poultry is influenced by the availability of serum TG. The majority of fatty acids are produced in the liver and are transported through LDL or cholesterol (Hermier, 1997). In contrast, HDL promotes the uptake of cholesterol from peripheral tissues and facilitates its transport to the liver for catabolism (Miller and Miller, 1975).

The greater reduction in serum lipid metabolites with feeding BAs alone or with lipase indicates an overall improvement in fat utilization, thus decreasing cholesterol synthesis and lipid deposition in the liver. It has been reported that BAs play a significant role in fat metabolism by emulsifying fats into smaller particles that enhance their interaction

with lipase (Lefebvre et al., 2009). Also, BAs regulate genes involved in lipid and cholesterol metabolism through pathways like FXR α and the TGR5, which influence cholesterol synthesis and uptake (Martinot et al., 2017; Portincasa et al., 2020). These regulatory effects can improve nutrient digestibility and absorption and reduce hepatic lipid deposition in animals fed diets supplemented with BAs. The hepatic expression of these receptors was increased in geese supplemented with BAs (150-300 mg/ diet) (Li et al., 2023). Supplementing BAs at 200 mg/kg diet has been shown to reduce TG and lipogenic genes in the liver of heat-stressed broilers (Yin et al., 2021b). Additionally, Liu et al. (2024b) indicated that supplementing BAs at 250 mg/kg diet reduced TG and fatty acid synthesis as well as fatty acid transport in the liver of broilers under corticosterone stress. Moreover, BAs feeding has been shown to decrease serum TG, TG and non-esterified fatty acids in broilers fed basal and high-fat diets (Hu, et al., 2024b). Similarly, Li et al. (2023) reported a reduction in serum TG and TG in geese supplemented with BAs (150-300 mg/kg diet). Also, lipase supplementation (0.15-0.3 g/kg diet) has been shown to reduce TG and LDL cholesterol as well as abdominal fat in broilers fed low-energy diets (Hu et al., 2018). In contrast, Lai et al. (2018a) indicated that supplementing broiler diets with BAs (40-80 mg/kg die) didn't affect serum TG, LDL and HDL. Also, BAs plus lipase supplementation did not affect serum lipid metabolites in broilers fed energy-reduced diets (Arshad et al., 2020; Shoaib et al., 2021). Regardless, the inconsistent or selective actions of BAs on lipid metabolism in various studies could be attributed to variations in dietary composition, experimental conditions and the composition, doses and sources of BAs used. Further studies are warranted to clarify the mechanisms involved in the BAs' actions on lipid metabolism in poultry and livestock. Regarding the OTC effect on serum lipid metabolites, it did not differ from the control. Previous research indicated inconsistent effects of OTC on lipid metabolism in poultry. Adding OTC to drinking water was associated with a reduction in egg yolk cholesterol and TG (Shaddad et al., 1985). In contrast, broilers fed OTC at 50 mg/kg showed greater levels of serum TC, LDL cholesterol and TG compared to the control (Abdullah, 2008).

Also, it was proposed that OTC administration to 3-month-old layers male chickens adversely affected liver functions as was reflected by the increased activity of enzymes indicating liver injury or stress (ALT, AST, GOT and GPT) (Siddig, 2014). Nevertheless, studies on the impact of OTC growth promoters on lipid metabolism are limited, and

considering current study results, BAs alone or with lipase are potential alternatives to OTC, not only as growth promoters but also as protectors of liver health and function.

4.3.2 Blood Protein and Immunity Parameters

As shown in Table (4.12), there were significant differences among treatments in serum total protein, WBC and IgG levels ($P < 0.05$). The BAs alone or with lipase showed the highest values for serum total protein and IgG, compared to both OTC and control treatments. The WBC was greater in the BAs group compared to other treatments. Notably, the lowest IgG levels were observed in broilers supplemented with OTC compared to other treatments. There was no significant difference in albumin levels among treatments.

Table 4. 12. Blood protein profile and immunity parameters of broiler supplemented with BAs, lipase and OTC

Treatment	Item			
	Total protein g/dl	Albumin g/dl	W.B.C *10(3)/ μ L	IgG nmol/ml
Control	B 2.40	A 1.52	B 21.22	B 210.48
OTC	B 2.41	A 1.49	B 21.11	C 204.73
BAs	A 2.79	A 1.51	A 21.63	A 213.91
BAs-L	A 2.83	A 1.52	B 21.55	A 214.48
SEM	3.44	1.10	3.14	3.17
P-value	0.012	0.52	0.014	0.011

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

The greater levels of serum total protein with BAs alone or with lipase supplementation indicated an improvement in protein synthesis and greater amino acid availability resulting from enhanced protein digestibility and utilization. Shoaib et al. (2021) reported a higher protein digestibility in broilers fed a low-energy diet supplemented with BAs plus lipase. It has been proposed that BAs enhance protein digestion and assimilation by activating the pancreatic proteases (Gass et al., 2007), in addition to enhancing digestion and absorption of dietary fats which generally increase the overall availability of nutrients. Our results agree with the results obtained by Hussien et al. (2022) who indicated a significant increase in total protein with no changes in albumin in broilers fed BAs (200 g/ton, Runeon®). Accordingly, the enhanced fat, protein and overall nutrient utilization contributed to the greater IgG levels induced by feeding BAs alone or plus lipase in our study. The higher IgG values imply a role of BAs and lipase in enhancing the immune response and overall health of broilers. In our study, the relative weights of the spleen and bursa of Fabricius were increased with BAs and lipase supplementation and these organs are involved in immune responses (e.g., antibody production, leukocyte maturation, etc), confirming a potential role of BAs and/or lipase in enhancing immune defense in broilers. Studies linking IgG levels to BAs and lipase supplementations are limited, but some studies indicated an overall improvement in immune response and health of broilers supplemented with BAs and lipase, particularly in heat stress conditions (Yin et al., 2021a; Yin et al., 2021b), aflatoxicosis (Chen et al., 2023) and intestinal inflammation (Bansal et al., 2020; Bansal et al., 2021).

The increase in WBCs can be attributed to promoting immune cell production, which leads to a higher WBC count, crucial for the immune response against pathogens. A previous study reported an increase in WBC in fish supplemented with BAs of the same composition used in our study but at a different dose (130 mg/kg feed) (Abdel-Tawwab et al., 2023). In contrast, Shoaib et al. (2021) indicated that feeding BAs alone or with lipase did not affect total protein, albumin and WBCs in broilers fed low-energy diets.

4.3.3 Liver and Kidney Function Parameters

Table (4.13) shows the changes in liver and kidney function parameters as affected by treatments. The ALT was lowest in broilers fed BAs plus lipase preceded by those fed BAs alone, OTC, and control, respectively ($P < 0.05$). For AST, the lower values were observed in broilers fed BAs alone or with lipase compared to OTC and control ($P < 0.05$). There was no significant difference in AST between BAs vs BAs plus lipase or between control and OTC ($P > 0.05$). The total and direct bilirubin didn't change with treatments.

The creatinine was decreased by BAs plus lipase compared to BAs, OTC and control ($P < 0.05$). The creatinine in broilers supplemented with BAs alone did not differ from control broilers ($P > 0.05$). The uric acid was decreased by BAs alone or with lipase compared to both OTC and control ($P > 0.05$).

Table 4. 13. Liver and kidney function parameters in broilers supplemented with BAs, lipase and OTC

Treatment	Item					
	ALT (u/l)	AST (u/l)	Total Bilirubin (mg/ dl)	Direct Bilirubin (mg / dl)	Creatinine (mg/ dl)	Uric Acid (mg / dl)
Control	A 10.26	A 256.82	A 0.87	A 0.13	A 0.54	A 5.45
OTC	B 9.68	A 242.10	A 0.82	A 0.12	A 0.55	A 5.66
BAs	C 5.77	B 203.04	A 0.85	A 0.10	A 0.53	B 4.61
BAs-L	D 5.33	B 204.06	A 0.83	A 0.11	B 0.51	B 4.57
SEM	2.59	2.20	3.20	2.19	3.44	3.22
P-value	0.022	0.021	0.30	0.11	0.011	0.011

- Means within the same column with different litters are significantly different at ($P < 0.05$).
- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

The reduced activity of AST and ALT by BAs alone or with lipase indicates enhanced liver function and health with these additives in our study and highlights the role of co-supplementation of BAs and lipase in protection against liver dysfunctions. The intermittent addition of BAs to broilers did not change ALT and AST at d 21 but decreased AST at d 35, as indicated by Hu et al. (2024b). Also, there was a linear decrease in both AST and ALT with increasing levels of BAs (from 0 to 350 mg/kg diet) in mullet diets. Similarly, BAs diminished the increase in AST and ALT associated with fatty liver hemorrhagic syndrome in laying hens fed high-fat diets (Li et al., 2024). Furthermore, BAs (250 mg/kg diet) have been indicated to alleviate the aflatoxin B1-induced liver injury in broilers fed BAs for 20 days (Chen et al., 2023). Additionally, Lai, et al. (2018b) reported a significant decrease in AST and ALT in broilers fed an 80 mg/kg diet of BAs, but they did not differ from control when feeding BAs at high doses (400 mg/kg diet). The bioavailability of OTC in poultry is relatively low and mainly exerts its action on gut microbiota (Ziółkowski et al., 2019), and this interprets its negligible role in improving liver functions in this study. The total and direct bilirubin levels in broilers fed BAs alone or with lipase did not differ from those in control indicating no adverse effects on liver functions and bile flow by using these additives. The lower levels of creatinine in BAs plus lipase treatments as well as the reduced levels of uric acid in BAs alone or with lipase treatments indicate proper kidney functions and enhanced protein utilization by these additives. In contrast to our results, Mohamed et al. (2020) reported increased ALT, AST and creatinine levels in broilers fed varying levels of bile salts (0.5, 1- and 1.5-mL bile salts/kg diet), but, similar to our results, there was no change in uric acid and bilirubin (total and direct). Also, Lai et al. (2018b) reported no change in creatinine, urea nitrogen, and bilirubin (total and direct) in broilers fed 80 or 400 mg/kg of BAs compared to the control. Thus, supplementing BAs alone or with lipase can reduce metabolic stress on liver and kidney functions, in addition to enhancing fat digestion, overall nutrient absorption and growth performance, thereby positioning BAs as a potential alternative to OTC as a growth promoter.

4.4 Digestive Enzymes

Table (4.14) shows the activity of digestive enzymes (amylase and lipase). They differed significantly ($P < 0.05$) among treatments. The higher amylase level was observed in broilers fed BAs alone followed by those fed BAs plus lipase compared to OTC and control groups. Lipase activity was greater in both BAs and BAs plus lipase treatments compared to both OTC and control treatments. There was no significant difference in amylase and lipase activity between OTC and control treatments ($P < 0.05$).

Table 4. 14. Serum levels of amylase and lipase enzymes in broiler supplemented with BAs, lipase and OTC

Treatments	Item	
	Amylase (u/l)	lipase (u/l)
Control	C 102.26	B 19.70
OTC	C 102.64	B 19.87
BAs	A 144.61	A 30.30
BAs-L	B 127.78	A 29.14
SEM	3.70	3.66
P-value	0.011	0.012

- Means within the same column with different litters are significantly different at ($P < 0.05$).

- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

The greater activity of amylase and lipase with supplementing BAs alone or with lipase indicates their roles in enhancing energy and fat utilization and better availability of

nutrients for overall body growth and health. In this study, the protease was not measured but previous research indicated an increase in pancreatic protease activity with the administration of BAs (Gass et al., 2007; Mohamed et al., 2020). These results agreed with those of Lai et al. (2018) and Ge et al. (2019) who indicated that supplementation with BAs at 60 and 80 mg/kg significantly increased the activity of duodenum lipase and lipoprotein lipase in broilers. Also, our results agreed with Hussien et al. (2022) who concluded that the action of intestinal lipase was amplified significantly due to the BAs fortification (200 g/ton, (Runeon®) of broilers' diets. Similarly, the activity of digestive enzymes amylase, lipase and protease was significantly increased with bile salts supplementation (0.5-1 ml/kg diet) to broilers diets (Mohamed et al., 2020). Recently, BAs supplementation (250 mg/kg diet) to high-fat diets was associated with greater lipase activity in the intestines of broilers (Chen et al., 2024). The increased activities of these enzymes enhanced the digestion and absorption of carbohydrates, fats, and protein, as reflected by the overall improvements in growth performance and feed efficiency metrics in the current study.

4.5 Cecal Total Bacterial Counts

Table (4.15) shows the level of cecal TBCs in broilers supplemented with BAs, lipase and OTC. The TBCs were significantly increased in the control group followed by BAs and BAs plus lipase groups and the lowest TBCs were observed in the group supplemented with OTC ($P<0.05$).

Table 4. 15. Total bacterial counts in cacal content of broilers supplemented with BAs, lipase and OTC

Treatment	Total Bacterial Counts (log CFU g)
Control	A 2.29
OTC	C 2.05
Bas	B 2.11
BAs-L	B 2.12
SEM	3.29
P-value	0.012

- Means within the same column with different litters are significantly different at ($P < 0.05$).
- OTC: oxytetracycline, BAs: bile acids, BAs-L: BAs plus lipase. SEM: standard error of means

A major decline in cecal TBCs was observed in broilers supplemented with OTC as a growth promoter. OTC inhibits protein synthesis in bacterial cells, suppressing harmful bacteria's growth in the intestine (Pickens and Tang, 2010). However, as a broad-spectrum antibiotic, it also may decrease the abundance of some beneficial bacteria (Smith et al., 2014), and this might be the reason for the significant reduction in cecal TBCS observed in broilers supplemented with OTC compared to other treatments. This was also reflected in the decreased growth performance and feed efficiency in broilers supplemented with OTC in the current study. Generally, APGs have been reported to reduce the activities of beneficial bacteria like lactobacillus which may disrupt intestinal health in the long term (Geng and Lin, 2016; Smith et al., 2014). In contrast, Shah et al. (2022) indicated that the abundance of gut-beneficial bacteria was not adversely affected by supplementing 100 or 200 mg/kg of OTC in a broiler diet. Nevertheless, the emergence

of antibiotic-resistant strains is a significant concern in poultry health and food safety (Pokrant et al., 2021; Pokrant et al., 2023).

The decreased cecal TBCs in broilers supplemented with BAs alone or with lipase compared to the control are attributed to their role in enhancing overall nutrient digestion and absorption and providing a less favorable environment for the development of pathogenic bacteria. Additionally, BAs have been shown to protect against intestinal absorption of bacterial endotoxins (Kocsár et al., 1969). A similar reduction in TBCs was observed in broilers fed bile salts (0.1-1.5 ml/kg diet) compared to the control (Mohamed et al., 2020). Also, BAs supplementation (200 mg/kg) has been shown to restore gut microbiota homeostasis in broilers under heat stress (Yin et al., 2021a). Moreover, it has been shown that DCS, a secondary bile acid added at 1.5 g/kg diet, reduced the counts of *Campylobacter junini* and firmicutes and increased Bacteroidetes count in broilers' intestines (Alrubaye et al., 2019). Recently, supplementing BAs to broilers fed high-fat diets resulted in reducing the abundance of Bacteroides while increasing those of Bifidobacterium, Firmicutes, and Lactobacillus, and these alterations in cecal microbiota populations were associated with an alleviation of fatty liver induced by feeding high-fat diets (Hu et al., 2023; Wang et al., 2024a). Furthermore, BAs (350 mg/kg diet) were proven to protect the intestinal barrier and reverse gut dysbiosis caused by *Salmonella Typhimurium* infection compared to antibiotic treatment in broilers (Hu et al., 2024a). Additionally, it has been indicated that BAs supplementation (150-300 mg/kg diet) was associated with higher abundances of gut microbes that produce short-chain fatty acids (SCFA); essential components for maintaining gut health and immunity (Li et al., 2023). These studies, as well as our study, indicate that BAs can modulate the gut microbiota diversity, and further research is warranted to clarify which microbiota are decreased /increased and at which dose of BAs under various dietary and experimental conditions.

Generally, it has been reviewed that BAs have antimicrobial functions and can modulate the gut microbiotas composition through various mechanisms including changing water solubility in bacterial cell walls, eliciting oxidative damage to bacterial DNA and RNA, or working as signaling molecules (via activating FXR and TGR5 receptors) to regulate the gut microbiota functions (Jia and Dong, 2024), thus inhibiting bacterial growth and proliferation and maintaining intestinal barrier integrity. The cecal TBCs were

numerically higher with BAs plus lipase vs BAs alone, indicating a potential synergistic effect of both BAs and lipase on gut microbiota modulation.

Based on the key findings of this study in terms of BWG and feed efficiency, carcass yield, immune organ relative weights, serum lipid metabolites, liver function enzymes, digestive enzymes and cecal TBCs, the co-administration of both BAs and lipase significantly improved these parameters compared to OTC. Therefore, the combination of BAs and lipase represents a nutritional viable strategy for enhancing broiler chicken production. Additionally, BAs serve as effective substitutes for APGs in broiler production. However, further studies are needed to investigate the underlying mechanisms of BAs, both alone or in combination with lipase from different sources and at varying doses to understand how these supplements can enhance the health and performance of broiler chickens reared under diverse dietary and experimental conditions.

CHAPTER V

CONCLUSION

Recently, BAs have been used as a dietary emulsifier in poultry production. Using BAs during the early stages of chickens has more potential to improve fat digestion and absorption than older ones. Exogenous lipase can potentially enhance the physiological efficiency of the poultry digestive system. However, little information is available on the synergistic effects of feeding BAs plus lipase on broilers' performance and metabolism. Additionally, antibiotics have been widely utilized in animal feeds, primarily to promote the growth of animals and to prevent bacterial infection. However, the misuse or overuse of antibiotics in animal production has resulted in the emergence of antimicrobial resistance posing a growing global threat to both human and animal health. Thus, there is an increasing interest in limiting the use of antibiotics as growth promoters and substituting them with safe alternatives. Due to their antimicrobial properties and capacity to modulate gut microbiota, BAs are emerging as potential alternatives to APGs. Studies directly comparing the efficacy of BAs to AGPs in terms of growth performance and feed efficiency are unavailable. Therefore, this study evaluated the effects of dietary supplementation of exogenous BAs alone or in combination with lipase on growth performance, feed efficiency, metabolic profile, liver health, immune response, and carcass characteristics in broilers. Also, these effects of BAs were compared to those of the antibiotic growth promoter OTC to explore their potential as substitutes for AGPs.

This study was conducted on Güntaş Tavukçuluk farm at Hamzabeyli Mahallesi Hamzabeyli sokak no: 80 Toroslar Mersin. The farm consists of two barns with a total capacity of 80,000 birds. It operates on a closed-rearing system with a precise electronic system controlling temperature and cooling. The poultry house was provided with thermostats and electrical heaters to get an appropriate temperature for each stage of growth, and the temperature was automatically controlled at 30-33, 28-29, 25-27, 23-24, and 21-22 °C at the first, second, third, fourth, and fifth weeks, respectively.

Two hundred forty Ross 308 broiler chickens were assigned to four treatments with four replicates of 15 birds each. The treatments included 1) a control group, receiving basal diet only; 2) an antibiotic group: received basal diet plus OTC as growth promotor at a dose of 100 mg/kg feed; 3) the BAs group: received basal diet plus BAs at a dose of 0.27

g/kg feed for weeks 1 and 2 and 0.25 g/kg feed for weeks 3, 4, and 5; and 4) Lipase-BAs group (BAs-L): received basal diet plus BAs as mentioned in the BAs group plus lipase at 0.18 g/kg feed. All birds had free access to water and feed. Commercial broiler feed, obtained from a commercial company (Şenpiliç), was fed to all birds as a basal diet according to the breed management book of Ross Company (AVIAGEN 2023) over three stages. Three diets were fed: starter for the first two weeks, grower for the next two weeks, and finisher for the final week.

Birds were individually weighed at 1,7,14, 21, 28, and 35 days old, and FI was calculated weekly until the end of the experiment's period on a replicate basis. The FCR, PER, EFR and EPEF were calculated accordingly. At the end of the experiment, three birds from each replicate of each treatment were slaughtered and the carcass yield, weights of the carcass' major parts (thighs, breast), and the relative weights of internal organs were measured. The pH, moisture and cooking loss were measured in breast muscle. Blood samples were obtained on the final day of the experiment (d 35) from 12 birds from each treatment (3 birds from each replicate) from the wing vein. The following blood parameters were measured: immune response (albumin, WBC, and IgG), lipid metabolites (TG, HDL, LDL, cholesterol), The liver (ALT and AST, Bilirubin) and kidney (uric acid, creatinine) functions indices, and digestive enzyme (amylase, lipase) activity. At the end of the experiment and after the birds were slaughtered, the cecal fresh contents from 12 birds of each treatment group were collected to measure the TBCs in cecal contents. Data were statistically analyzed using SPSS 18 version software and the Duncan Multiple Range Test was applied to detect the differences among treatments ($P < 0.05$).

The key findings of the current study are:

Broilers supplemented with BAs showed the highest body weight in the 2nd week ($P < 0.05$) while the broilers fed BAs-L showed the highest body weight in the 5th week ($P < 0.05$), compared to OTC and control groups. During the first week of age, results indicated that higher BWG was observed in BAs-L and OTC groups compared to both control and BAs groups ($P < 0.05$). In the 5th week and the whole cumulative period, the increase in BWG was recorded for the BAS-L group followed by the BAs group compared to both OTC and control groups ($P < 0.05$). Dietary supplementation of BAS-L and BAs showed lower FI during the 5th week and the whole period compared to the

OTC and control groups ($P < 0.05$). In the first week, broilers fed BAs-L showed enhanced FCR followed by those fed BAs alone whereas, in week 2, broilers fed BAs showed an improved FCR followed by those fed BAs-L, compared to control and OTC treatments ($P < 0.05$). For the 4th and 5th weeks as well as the whole cumulative experimental period, the enhanced FCR was observed in broilers supplemented with BAs and BAs-L compared to both control and OTC groups ($P < 0.05$). The highest EER and EPEF values were recorded for both BAs and BAs-L treatments compared to both OTC and control groups ($P < 0.05$). The OTC treatment did not differ from the control in growth performance and feed efficiency metrics except in week 1, which showed a lower FI and higher EFR but in week 2 it showed a lower EFR than the control.

The live weight, hot carcass weight, cold carcass weight and dressing percentage differ significantly ($P < 0.05$) among treatments with the highest values observed in BAs and BAs-L groups compared to control and OTC-supplemented groups. The results indicated significant differences ($P < 0.05$) among treatments in various organs except for heart, liver and intestinal weight. Compared to other treatments, the highest breast, thigh, bursa, and spleen and the lowest abdominal fat relative weights were observed in broilers fed BAs alone or with lipase. Feeding of OTC produced the lowest relative weight for thigh muscles and the highest gizzard relative weights compared to other treatments ($P < 0.05$). The meat cooking losses were greater for BAs alone or with lipase, while the pH and moisture % did not significantly change compared to control and OTC treatments.

Broilers supplemented with BAs alone or with lipase showed significantly lower values ($P < 0.05$) for serum lipid metabolites (cholesterol, HDL, LDL, and TG), liver function enzymes (ALT and AST), and uric acid compared to broilers fed the control and OTC-supplemented diets. Also, BAs alone or with lipase supplementation significantly increased ($P < 0.05$) the serum total protein and IgG levels as well as digestive enzymes (amylase and lipase) activity compared to broilers fed the control and OTC-supplemented diets. Notably, the lowest IgG concentrations were recorded in broilers supplemented with OTC compared to other treatments. The creatinine levels were decreased by BAs plus lipase supplementation compared to BAs, OTC, and control treatments ($P < 0.05$). The creatinine in broilers supplemented with BAs alone did not differ from control broilers ($P > 0.05$). The TBCs were significantly increased in the control group followed

by BAs and BAs plus lipase groups and the lowest count was observed in the group supplemented with OTC ($P<0.05$).

Therefore, the overall findings confirmed the efficacy of BAs as an alternative to the OTC growth promoter in promoting the growth and health of broilers. The results suggest that while the addition of lipase to a diet containing BAs did not consistently produce significant differences compared to feeding BAs alone, there were notable variations in specific metrics. Broilers supplemented with BAs plus lipase exhibited better BWG and FCR in 1st week and had higher relative weights of the Bursa of Fabricius and gizzard and lower relative abdominal fat compared to those fed only BAs. Conversely, broilers receiving only BAs demonstrated high values for EPEF, WBCs, ALT, creatinine and greater amylase than those supplemented with BAs plus lipase. These findings indicate that while combining BAs with lipase may offer some benefits, it does not universally enhance all performance metrics when compared to BAs alone. Therefore, further studies are needed to determine the optimal doses of both BAs and lipase, both individually and in combination to understand their synergistic effects on broiler health and performance at various stages.

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CURRICULUM VITAE

Ben Tarek Diab, ... doğumluyum. Lise eğitimimi Suriye'nin Homs şehrinde tamamladım. 2007 yılında Suriye Veterinerlik Fakültesi'nden mezun oldum ve eğitimim sırasında çeşitli uluslararası projelerde ve konferanslarda aktif olarak yer aldım.

Mezuniyetimin ardından, babama ait olan ve babamın da veteriner olduğu bir yumurta tavukçuluğu çiftliğini yönettim. Daha sonra, Şam'daki Tarım Bakanlığı'na atandım ve burada Viral Aşı Üretim Departmanı'nda çalıştım. Ardından, beş yıl boyunca Viral Kanatlı Aşı Üretim Departmanı Başkanı olarak görev yaptım.

Tarım Bakanlığı'ndaki görevim sırasında birçok sorumluluk üstlendim. Hindistan'dan et ithalatını denetlemek üzere Suriye heyeti başkanı olarak görevlendirildim ve ayrıca Veteriner İlaç Teknik Komitesi ile Hayvancılık İşleri Danışma Komitesi üyesi olarak görev yaptım.

Türkiye'ye taşındıktan sonra, Mersin'de 400.000 kuş kapasitesine sahip bir yumurta çiftliğinde beş yıl boyunca çalıştım. Daha sonra, yem katkı maddeleri üretiminde uzmanlaşmış bir şirket olan ADEMİX'te Teknik ve Bilimsel Destek Bölüm Başkanı olarak görev aldım. Şu anda biyoteknoloji araştırmaları ve aşı üretimi alanında faaliyet gösteren DOLLVET'te çalışmaya devam ediyorum.

