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EFFECT OF NATURAL AND SYNTHETIC ANTIOXIDANT AS FEED ADDITIVES ON BROILER CHICKENS AND CONSUMER HEALTH

By

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ABSTRACT

This study was carried out to investigate the potential effect of natural antioxidant (edible mushroom *Agaricus bisporus* and vitamin E & Selenium Se) and synthetic antioxidant butylated hydroxytoluene (BHT) as dietary supplementation to broiler chicken on growth performance, meat lipid profiles and residual detection of BHT in raw and cooked meat (liver, thigh and breast tissues) by boiling as well as the histopathological changes of these antioxidants on chicken liver and muscles. One hundred one - day old unsexed broiler chickens, divided randomly after one-week age into four equal groups. The first group (Gp1) kept as control fed on basal diet without any supplementation, the second group (Gp2) fed on basal diet supplemented with dried mushroom at a dose 1.5 gm / kg ration, the third group (Gp3) fed on basal diet supplemented with vitamin E and Selenium (Vit. E & Se) in a dose 100 mg/kg ration and the forth group (Gp4) fed on basal diet supplemented with butylated hydroxytoluene (BHT) at a dose 100 mg/kg ration. Two types of specimens were taken from liver, thigh and breast tissues of all groups at slaughter time (42 day of age).

Data obtained on growth performance were significantly increased in mushroom and vit E&Se supplemented groups and significantly decreased in BHT supplemented group. The lipid profile of meat showed significantly decreased in all experimental groups while significantly increased in liver cholesterol and low-density lipoprotein (LDL) of (BHT) supplemented group compared with control. Residual detection of BHT was high and significantly difference in raw tissues (liver, thigh and breast) than the cooked tissues which undergo boiled at 80^{O}C for 30 minutes, and the reduction percent of BHT in cooked meat (liver, thigh and breast) were 46.80, 54.74 and 94.53, respectively. No obvious pathological

alteration observed in mushroom and vit. E&Se supplemented groups. Congestion of hepatic blood vessels with portal and interstitial round cells aggregation were observed in liver, in addition hyaline degeneration with interstitial edema were also seen in skeletal muscles in (BHT) supplemented group.

Therefore, antioxidants can be added to the ration to revert the deleterious effects of lipid oxidation in poultry production which affects both feedstuffs and poultry.

I*n vivo*; subsequently, oxidation affects the quality and shelf life of poultry products for human consumption. It seems also that mushroom and vit,E&Se may be beneficial components as natural antioxidants in broiler diet than the synthetic antioxidant BHT.

Key words:

Broiler, Mushroom,Vitamin E and Selenium (Se), Butylated hydroxytoluene (BHT), liver and muscles tissue.

INTRODUCTION

Poultry meat is a very popular commodity around the world and its production and consumption have been increasing rapidly in the last few decades. Poultry meat is preferred by consumers due to low production cost, low fat content and high nutritional value **(Khiari *et al.,* 2014 and Tuba Candan and Aytunga Bagdath, 2017).**

In general, broilers are subjected to various stresses, which include heat stress, nutritional stress due to dietary nutritional imbalance, overcrowding stress and handling stress. High stocking density (HSD), along with high environmental temperature, may induce oxidative stress in birds as rate body temperature may be relatively high when compared to mammals. Poultry can only express their full genetic potential when reared under thermo neutral temperatures **(Cassuce *et al.,* 2013 and Selvam *et al.*, 2017).**

Overcrowding in HSD may have negatively effect on broiler performance and health. Moreover, heat stress activates hypothalamic-pituitary-adrenal (HPA) axis and increases corticosterone levels of chickens, resulting in reduced feed intake, body weight gain, relative immune organ weight and innate immunity **(Quinteiro-Filho *et al.*,2010 and Selvam *et al.*,2017).**

Antibiotics are widely used as growth promoters in poultry production. In recent years, usage of antibiotics as growth promoter in poultry diet has been banned due to concerns about their residues in animal tissues and subsequent induction of emerging antibiotic resistant strains

(Simon, 2005; Salah *et al.,* 2009 and Abolfazl *et al.,* 2014a). Therefore, researchers are looking for safe alternative's candidates such as natural products and phytobiotics. Recently, natural materials such as medicinal products originating from fungi or herbs have been used in animal feeding to improve performance through amelioration of feed properties, promotion of production performance, and improving the quality of meat of animal origin **(Toghyani *et al.,*2010; Abolfazl *et al.,*2014a and Ashkan *et al.,* 2014).**Antimicrobial activities, immune enhancement and stress reduction in farm animals given from natural medicinal products from fungi and herbs **(Abolfazl *et al.,* 2014a and Ashkan *et al.,* 2014)**. Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value.

The fungi have a wide range of activities and have been used for centuries to combat disease outbreaks in many parts of the world such as Asian and Mediterranean countries **(Guo *et al.,* 2003 and Ashkan *et al.,* 2014).** Mushroom and its different derivatives contain a variety of active substances like ergothioneine**,** phenolic antioxidants,variegatic acid and dibiviquinone **(Kasuga *et al.,* 1995 and Ashkan *et al.,* 2014).** However, there have been few reports on the effect of *Agaricus bisporus* mushroom in chickens.

It has been hypothesized that some of the properties of plants or fungi are carried through a prebiotic effect due to their polysaccharide content **(Guo *et al.,* 2003; Ashkan *et al.,* 2014 and Daniel Spoljarie, 2015).**

Oxidation is a result of natural metabolic processes, but excessive formation of reactive oxygen substances (ROS), such as free radicals, which can damage important biomolecules (lipids, proteins, and nucleic acids) in the body of human and animals.

The rate of oxidation increases as a result of the following: (1) high intake of oxidized lipids and prooxidants; (2) deterioration of sensitive polyunsaturated fatty acids (PUFA); and (3) low intake of antioxidative nutrients. In muscles tissues oxidative reactions continue postmortem and are a leading cause of quality deterioration during processing and storage. With a relatively high proportion of PUFA, poultry meat is more susceptible to oxidative processes, specifically lipid oxidation, than beef or pork. Therefore, incorporation of dietary antioxidants, such as vitamin E and Se in poultry feed, has been implemented to achieve optimal growth performance, reproduction and meat quality **(Smet *et al.,* 2008 and Rebecca *et al.*,2014).** Many of natural antioxidants are provided with the chicken diet (vitamin E, carotenoids, selenium, etc), while a range of other antioxidant compounds are synthesized in the body (glutathione,thioredoxins,antioxidant enzymes,etc) and a delicate balance between

antioxidants and pro-oxidants in cells, digestive tract and in the whole body is responsible for maintenance of chicken health, their productive and reproductive performances **(Fotina *et al.*, 2013)**.Natural or synthetic antioxidants are usually used to slow down or stop lipid peroxidation and in consequence to preserve freshness of the product. Many natural antioxidants, such as tocopherol, vitamin C, flavonoids, for a short period, may be effective in food preserving, but in many cases such protection is not sufficient. Therefore, synthetic antioxidants are widely used,among which BHT (Butylated hydroxytoluene);BHA (Butylated hydroxyanisole) and EQ (ethoxyquin) are the most frequent. **(Alina Blaszczyk *et al.*, 2013)**. Therefore, the present study was designed to investigate the effect of dietary supplementation of natural antioxidants (mushroom and vitamin E& Se) and synthetic antioxidants (BHT) on broiler growth performance, consumer health in the form of carcass characteristics (oxidation status of liver, thigh and breast) and residue of (BHT) in meat as well as the effect of these antioxidants on the histopathological changes of chicken's liver and muscles.

MATERIAL AND METHODS

Natural Antioxidant:

-Mushrooms:

The commercial *Agaricus bisporus* mushroom was purchased from a local mushroom producer and the dryness process was carried out in Agriculture Collage Tanta University to obtained the dried powder.

-Vitamin E and Selenium:

Vit. E&Se was obtained from BS Pharma Com. Industrial area (6A) 10 Ramadan city.

Synthetic antioxidant:

-Butylated hydroxytoluene (BHT):

2, 6-Di-Tert-Butyl-Hydroxytoluene were purchased from Sigma Co. (Egypt).

Experimental Design:

One hundred unsexed one day old broiler chicks obtained from commercial hatchery. All chicks were kept under strict hygienic measures. Chicks provided with water ad libitum along the experimental period (42days) and fed ad libitum on balanced diet according to **(NRC 1994).** Chicks were housed in isolation units in a biosecure animal housing and reared on deep litter floor. After 7th day of age, chickens were randomly divided into four groups. The first group (Gp1) was kept as control fed on basal diet without any supplementation.

The second group (Gp2) was fed on basal diet supplemented with dried mushroom 1.5 gm/kg ration according to **(Giannenas *et al.,* 2010)**. The third group (Gp3) was fed on basal diet in addition to vitamin E and Se. 100 mg / kg ration according to ***(*Englmaierova *et al.,* 2011)**. The forth group (Gp4) was fed on basal-diet supplemented with butylated hydroxytoluene (BHT) 100 mg/kg ration according to **Barbara Nieva *et al. (*2015).**All chickens were vaccinated against (ND: at 7th day of age, Hitchner and 21th day of age Lasota strain) and against IBD Gumboro at 14th day of age.Live body weight, body weight gain **(Brady, 1968)**, feed consumption and feed conversion **(Ensminger 1980)** and performance index **(North 1984)** were monitored weekly along the experimental period (42 days) and any changes were recorded. After the end of 42 days, five broilers chickens were randomly taken from each group weighted and slaughtered. Necropsy was performed.

Two types of specimens were taken from liver, thigh and breast muscles. First specimens were preserved at -20°C for determination of cholesterol, triglyceride, lipoprotein in all groups beside residue of the BHT in BHT supplemented group (GP4), the second specimens were preserved in 10% neutral buffered formalin solution for histopathological examination.

Determination of meat lipid profile:

Biodiagnostic Company, Dokki, Cairo, Egypt (2018). Known quantity (50 gm) of meat was sampled from the liver, thigh and breast of dressed carcasses and analyzed to determine the amount of total cholesterol, triglyceride, High Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) using spectrophotometer according to the methods of **(Allain *et al.,* 1974).**

Determination of butylated hydroxytoluene by HPLC:

A known quantity of tissues (liver, thigh and breast) of dressed carcasses from BHT supplemented group were divided into two parts, the first one was analysed to determine the residue of BHT in raw tissues and the second part undergo boiling at 80°C for 30 minutes and analysed for detection BHT residue after cooking. Butylated hydroxytoluene (BHT) was extracted from the examined samples of chicken tissues using acetonitrile according to the method described by **(Lundbye *et al.*, 2010)** and was analyzed using High-Performance Liquid Chromatography (HPLC). The detection of BHT was performed at excitation 282nm and emission 307 nm. Further, BHT was quantified by HPLC method similar to that described by **(Yankah *et al.*, 1998)**, using a fluorometric detector (excitation = 290 nm/emission=233 nm) and both acetonitrile/methanol (50:50) and water/acetic acid (95:5) as mobile phases. The limits of quantification (LOQ) for the analytical methods for determining

the BHT was 5 mg/ kg. The recovery and analytical precision (in terms of relative standard deviation, RSD) were 104.9%, 87.1% and 83.5%, respectively.

Table (1): Recovery rate (%) of tested BHT in the examined broiler samples.

BHT	Recovery Rate (%)
Liver	**104.9**
Thigh	**87.1**
Breast	**83.5**

Pathological Examination:

After the end of the experimental period, necropsy was performed and tissue specimens from liver and muscles were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol concentrations (70-100), cleared in xylene and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (H&E) dyes according to **(Suvarna *et al.,* 2013)** and examined microscopically.

Statistical Analysis:

All data obtained were analyzed using ANOVA (**SAS 2010).** Significant differences among treatments were determined by **Duncan, (1955)**.

RESULTS AND DISCUSSION

All experimental groups showed no abnormal clinical signs along the whole experimental period. No mortality was observed throughout the experiment.

The live body weight, body weight gain, feed intake, feed conversion ratio and the performance index of chicken in different groups during the experimental period are illustrated in (Table 2). The data showed significantly increase in body weight, weight gain, feed intake and the performance index in groups supplemented with natural antioxidants {Mushroom (Gp 2) and vit. E &Se (Gp3)} and significantly decrease in synthetic antioxidants BHT supplemented group (Gp 4)}.

The obtained results on body weight of mushroom supplemented group are in accordance with the results obtained by **Kavyani, *et al. (*2012);Abolfazl *et al. (*2014a) and Mazaheri *et al. (*2014),** they reported that, the mushroom composition in relation to physiochemical

properties, the phenolic compounds and polysaccharide fractions as well as sugar composition, molecular weights, and structures could be the basis for the observed results.

These data are partially consistent with **Giannenas, *et al.* (2010),** who showed no significant effects of body weight values by mushroom treatment up to 28 day of age but at 42day of age, the body weight, and body weight gain values were greater than the control.

In contrast to the obtained results, some reports showed that, the use of mushroom and mannan oligosaccharides had no effect on the feed intake **(Willis *et al.*, 2007; Yalcinkayal *et al.*, 2008 and Falaki *et al.*, 2011).** They suggested that under the beneficial management and/ or environmental conditions, the effect of such feed additives may be worthless. The discrepancy in these reports could be related to the differences in management, environmental conditions that exist in various experiments.

The obtained results on body weight of vit. E&Se supplemented group were agreed with **Hosseini Mansoub, *et al.* (2010); Ismail, *et al.* (2014) and Selvam, *et al.* (2017),** who found that dietary enrichment with vit. E resulted in better performance when compared to the birds fed with standard diet without vit. E. Also similar results were obtained by **Biswas, *et al.* (2011)** who explained that broiler treated with different levels of vit. E&Se showed increase in body weight gain than control group. The better performance might be due to the synergistic action of vit. E&Se on the physiological system of the birds. On the same line, **Khattak, *et al.* (2012)** reported that vit. E supplementation at 300mg/kg produced a better performance in broilers under heat stress. The present data related to body weight in BHT supplemented group were in a partial agreement with **Larsen, *et al.* (1985) and Barbara, *et al.* (2015)**, who recorded that chicken gave a diet containing eight times to the normal concentration of BHT, given form 1 day to 6 weeks old were similar in growth rates as control birds, on the other hand the body weight gain was less in birds given a diet containing 30, 80 times of BHT. Additional effects such as behavioral changes, reduction in body weight gain and decrement in body weight have been observed after long term administration of BHT to mice and rats **(Stokes and Scudder, 1974; Olsen *et al.*, 1986; Tanaka *et al.*, 1993; Price, 1994; JECFA, 1996 and Barbara *et al.*, 2015)**.

This difference in results may be attributed to the variation in management and environmental condition.

Tissue lipid profiles of broiler chickens in different groups are illustrated in (Table 3). All parameters were influenced by natural (mushroom and vit. E&Se) and synthetic (BHT)

antioxidants. Data showed significantly decrease in tissues triglyceride (P<0.05) in all experimental groups (mushroom, vit. E&Se and BHT) of all examined tissues (liver and thigh and breast meat) compared with the control group. Significantly decrease (P<0.05) in cholesterol in all experimental groups (mushroom, vit. E&Se and BHT) of all examined tissues (liver and thighv and breast meat) compared with the control group except the liver tissue of BHT supplemented group.

Significantly decrease (P<0.05) in LDL in all experimental groups of all examined tissues compared with the control group except the liver tissue of BHT supplemented group.

Also significantly decrease (P<0.05) in HDL in all experimental groups (mushroom, vit. E&Se and BHT) of all examined tissues (liver and thigh& breast meat) compared with the control group.

The present study in mushroom supplemented group lipid profile may go hand to hand with previous studies reported by **Abolfazl, *et al. (*2014b)** on serum Japanese quils and by **Ekunseitan, *et al. (*2017)** on meat of chicken.

The obtained results explained by **Thompson, *et al.(*1984); Fukushima, *et al. (*2001); Preuss, *et al. (*2007) and Abolfazl, *et al.(*2014b)** suggested that mushroom dietary fiber might bind bile acids to reduce their entry into enterohepatic circulation, which then lead to an increase in gut bile acid secretion. As a result, the liver responds by increasing hepatic conversion of cholesterol into bile acids, thus reducing its circulating levels.

In the present study lipid profile decreased in vit. E&Se supplemented group, these results are in accordance with **Ramnath, *et al. (*2008); Tan *et al.(* 2010); Halici, *et al. (*2012); Siegel and Gross, (2014) and Erol, *et al. (*2017),** they recorded that animal under stress, several changes occur in some metabolic parameters to ensure the adaptation of stress, some of these varying parameters are plasma concentration of glucose, total protein, triglyceride, cholesterol, non-esterified fatty acids and VLDL resulting an increase LPO ratio in liver and thigh and breast tissue and vit. E decreased LPO levels increased by stress in both liver, breast and thigh tissue. In alike manner **(Lin *et al.*,2006 and Bayraktar *et al.*,2011)** reported that, the metabolic effects under heat stress may differ from tissue to tissue leading to enhance the cholesterol and triglyceride amounts while vit. E tended to decrease them.

Selenium (Se) - an integral part of 25 selenoproteins participating in various antioxidant reactions in the body. It is supplied with the diet in various forms and optimal Se status is a

key for effective antioxidant protection **(Pappas *et al.,* 2008 and Fotina *et al.,* 2013)**. Vitamin E (ᾳ- tocopherol) is a hydrophobic antioxidant that scavenges the free radicals' hydroxyl, alkoxyl, peroxyl and superoxide anion through non-enzymatic defense **(Amaneh *et al.,* 2015 and Selvam *et al.,* 2017).** Vitamin E is considered a first line of defense against lipid peroxidation, preventing its chain propagation by quenching free radicals. It acts on the early stage of lipid peroxidation and enhances cell membrane stability.

Therefore, under stressful conditions, vitamin E supplementation in diet is necessary to protect tissues from lipid peroxidation. The supplementation of vitamin E to broilers is also an important factor for the health of human consuming chicken meat as it is known to improve meat quality by upregulating the expression of antioxidant enzyme genes broilers **(Adebiyi *et al.,* 2011; Niu *et al.,* 2017 and Selvam *et al.,* 2017).**

The lipid profile in BHT supplemented group in the present study showed significant decrease in lipid profile (triglyceride, cholesterol, LDL and HDL) in thigh and breast meat. Also, significant decrease in triglyceride and HDL of liver tissue but significant increase in cholesterol and LDL in liver tissue compared with control group. BHT undergoes biotransformational processes not only in foods, natural environments, such as air, dust, soils or water **(Fries and puttmann, 2004; Fernandez Alvarez *et al.,* 2009 and Rodil *et al.,* 2012)** but also in living organisms. The metabolism of BHT is very complex and it has been investigated in different animal species, such as rats **(Matsuo *et al.,* 1984 and Conning and Phillips, 1986),** rabbits **(Dacre, 1961 and Conning and Phillips, 1986),** chickens **(Frawley *et al.,* 1965**) and also in human **(Daniel *et al.,* 1968 and Conning and Phillips, 1986).**

As BHT undergoes several reactions during biotransformation, a large number of intermediate metabolites have been identified. However, their nature and concentration depend on the environmental conditions and on the animal species.

The results indicated that BHT and its toxic metabolite could remain bioaccessible for intestinal absorption. Due to the great importance of matter, further research on the potential oxidation process of BHT during digestion and on the nature of the metabolites arising from it will be needed **(Barbara Nieva *et al.,*2015).** BHT has been reported to efficiently react against strong oxidizing radicals such as singlet oxygen (O_2), hydroxyl radical (OH), and peroxyl radicals (OOR), although the mechanism of quenching reactions may vary depending on the reactive oxygen species (ROS) involved and the local environmental **(Lambert *et al.,* 1996).** Nevertheless, the antioxidant activity of this molecule at high temperatures can greatly

differ from that developed at moderate temperature and noticed lower antioxidant activity **(Zhang *et al.,* 2004 and Marmesat *et al.,* 2010).**

This fact was attributed not only to the compound volatilization but also to its transformation into metabolites. However, BHT has also reported to exert prooxidant effects under certain conditions. Thus, when BHT was added in excess to a wheat seedling medium in aerobic conditions, an enhancement of the generation rate of superoxide anion was observed.

This is a reactive particle that may damage cellular structures at high concentrations **(Smirnova *et al.,* 2002 and Barbara Nieva *et al.,* 2015).**

In addition, an increase in hepatic microsomal lipid peroxidation was observed in rats fed with diets containing 0.2% of BHT for 30 day **(Yamamoto *et al.,* 1995).**

Regarding to the residue of BHT in meat tissues on the present study, (Table 4) showed accumulation of the BHT in both raw and cooked tissues (liver, thigh and breast).

Our data showed the level of BHT residue in raw samples were more than in cooked samples which undergo boiling at 80ºC for 30 minutes also its accumulation increased in liver tissue than the thigh and the breast meat. The obtained results detected the synthetic antioxidant residue (BHT) in raw liver, thigh and breast were 4.70±0.20, 3.17±0.25 and 1.83±0.31 respectively otherwise the BHT residue in cooked live, thigh and breast were 2.53±0.35, 1.43±0.25 and 0.10±0.17 respectively.

The increasing level of BHT in raw meat than the cooked may be attributed to the effect of high temperature which cause volatilization and also steam distillation caused by water boiled out of the cooked meat, but also to their rapid degradation, these results with the same manner with **Ciênc, (2011) and Bitar *et al*. (2008)** they showed the thermal behavior of the synthetic antioxidants BHT and detected antioxidants BHT decompose or start their decomposition at temperatures below 100 °C. Thus, residual amounts of the parent compound and/or its metabolites may be ingested by humans from a wide variety of animal products. Although the maximum residue limits (MRLs) of BHT have been established as 10 mg/kg for fish, 3 mg/kg for chicken fat and 0.5 mg/kg for pig fat in some countries like Japan **(JMHLW 2005)**, in the European Union these (MRLs) do not currently exist for synthetic antioxidants in food products of animal origin **(Barbara Nieva *et al.,* 2015).**

While maximum residue limits (MRLs) of BHT in chicken tissues have been established as 200 ppm according to Egyptian organization for Standardization and Quality **(ES 2006)**.

The obtained results detected the synthetic antioxidant residue (BHT) in raw liver and thighs were more than MRLs according to **JMHLW, (2005) and ES, (2006).**

However, some effects of synthetic antioxidants are not always beneficial for our health **(Barbara Nieva *et al.,* 2015).** Antioxidants such as BHA or BHT have been widely used for many years to preserve freshness, flavor and colour of foods and animal feeds as well as to improve the ability of pharmaceuticals and cosmetics **(Alina Blaszczyk *et al.,* 2013)**.

On the same manner the results in (Table 4) explained the reduction percent of BHT in examined samples and illustrated the effect of heat and processing of meat by boiling, moreover the reduction percent in cooked liver, thigh and breast were 46.80, 54.74 and 94.53 respectively, and all samples became under permissible limit of BHT in chicken tissues according to **JMHLW (2005).**

While cooked liver still more than permissible limit of BHT in chicken tissues according to **ES (2006).** BHT is one of the most commonly employed food antioxidants and its use in Europe is restricted to different dosages**.** In addition to its use as a food antioxidant, BHT may also be added to animal feeds, food packing materials and pharmaceuticals. Due to the presence of BHT in the waste waters generated by these industries, it can also be found in natural environments like soil, water, and air. BHT can also reach the food chain by these means **(Barbara Nieva *et al.,* 2015).**

Only a few studies have addressed the fate of BHT in foods during processing at high temperature. **(Augustin and Berry, 1983; Allam and Mohamed, 2002; Tsaknis *et al.,* 2002 and Zhang *et al.,* 2004).** Some studies which focus on the accumulation of BHT in fatty tissues after feeding animals with diets containing BHT can also be found **(Frawley *et al.,* 1965 and Barbara Nieva *et al.,* 2015).**

They fed chicken with a diet containing 200mg/kg of BHT for 10 week and reported that their edible portion contain 1 and 3 mg/kg of BHT and its metabolites, and even higher concentrations were found in fat, skin, liver and viscera.

Pathological Findings:

By macroscopically examination:

Both liver and skeletal muscles of all groups were apparently normal except livers of BHT supplemented group were relatively large with rounded borders.

Microscopically:

1-Mushroom:

Examined sections from liver of group (Gp 2) which fed on basal diet supplemented with dried mushroom, showed apparently normal hepatocytes Fig.1 (A, B). Few sections showed degenerative changes in some hepatocytes mainly vacuolar and hydropic degeneration.

Cross and longitudinal sections of skeletal muscle from group (Gp 2) were apparently normal with preserved striations and peripherally located nuclei Fig.1 (C, D).

2- Vit. E and Selenium:

Examined sections from liver of group (Gp 3) which fed on basal diet supplemented with vit. E and Selenium, showed nearly normal histo-morphologic architecture of hepatocytes Fig.2 (A). Examined sections of skeletal muscle of group (Gp 3), were normal with preserved striations and interstitial tissue Fig.2 (B, C). A few muscle fibers showed hyaline degeneration with mild interstitial edema, Fig.2 (D, E).

3- BHT:

Examined sections from ***liver*** of group (Gp 4) which fed on basal diet supplemented with BHT. Showed congestion of hepatic blood vessels, portal and interstitial round cells aggregations together with mild biliary proliferation Fig.3 (A, B, C and D).

Cross and longitudinal sections of *skeletal muscles* were normal with preserved striations and interstitial tissue. A few muscle fibers showed hyaline degeneration with swollen deep eosinophilic fibrils, pyknotic nuclei and moderate interstitial edema.

The intermuscular arterioles and venules showed swollen endothelium and dilatation respectively beside perivascular edema. Congestion of the intermuscular blood vessels together with lymphocytic infiltration were also seen (E and F).

Concerning to our results in the histopathological alteration on liver and skeletal muscle, the liver and muscle in mushroom supplemented group (GP.2) were relatively normal. No available literature on the pathological changes of chicken fed on mushroom. Only few literatures were performed in rats by **Miranda, *et al.* (2014),** who reported that histological analysis of kidneys and liver tissue was performed. He suggested that there was no hepatotoxic effect caused by mushroom supplementation in diets.

On that contention **(Chen *et al.*, 2014)** on mouse, he recorded that basal diet supplemented with mushroom extract, the Histopathological observation indicated that, the extract of

mushroom could effectively prevent excessive lipid formation in liver tissue.

The pathological changes of the liver and muscle in vit. E&Se supplemented group (Gp.3), showed nearly normal hist-morphologic architecture. **Parker and Landvit (1990) and Ahmed, *et al.* (2015),** they explained the ability of vit. E in protecting liver from lipid peroxidation and keep cells membranes from damage.

This is results from the methyl groups of tocopherol that interact with the Cis double bounds of the fatty acids to form a stable complex in membrane phospholipids, this action leads to reduced MDA synthesis, thus decreasing its level in this organ **(Shahin *et al.*,2001).**

The macroscopical picture of the livers in BHT supplemented group were enlarged with rounded borders. Similar results are observed by **Rao, *et al.* (2000)** in chicken, he showed a marked congestion of the liver and kidneys, as well as diffuse enlargement of the liver with rounded borders and rupture with hemorrhaging.

Microscopically the livers in this group showed congestion of hepatic blood vessels with round cell aggregation, nearly the same results were recorded by **Rao *et al.* (2000).**

Also our results are in accordance with **Nakagawa, *et al.* (1984) and Nakagawa and Tayana (1988)** on rats. They concluded that acute hepatic damage and centilobular necrosis were observed. These findings are either due to the direct effect of BHT quinine methide produced by cytochrome P-450 linked monoxygenase system, this metabolite concentrated in liver and kidneys tissue. Also another observation showed a marked elevation of GPT which is an indicative enzyme for liver damage. On the same line our results are agreement with **Takahashi and Hiraga, (1982),** they reported a mild damage of the liver in rats given BHT. Studies performed on rats also reported a dose-related increases hepatocellular adenomas and carcinomas **(Olsen *et al.*, 1986).** Microscopically, skeletal muscles in BHT supplemented group in this study showed interstitial edema, congestion of blood vessels together with lymphocytic infiltration.

Our results are agreement with **Takahashi and Hiraga, (1982),** who reported that, the congestion and bleeding observed in BHT treated rats may be attributed to the intrinsic coagulation initiated by the activation of factor XII couldn't precede because of the decrease of factors IX, VIII, X and prothrombin which might be one of the effects enhancing bleeding tendency. The reduction in blood coagulation factors IX, VIII, VII and prothrombin in BHT treated rats might a stronger and central effect in BHT reduced hemorrhage.

On the same context **(Rao *et al.*, 2000)** confirmed these results by elevation in bleeding time, clotting time and prothrombin time in BHT treated broiler chicken.

CONCLUSION

The results presented in this study showed a growth promoting activity and an increase in the antioxidative capacity of broiler chicken tissues (low cholesterol and low triglyceride) as demanded by health-conscious consumers as a result of dietary mushroom and vit. E&Se supplementation with no pathological alteration while in BHT supplemented group some attention should be taken about its safety as feed additives for its residues in meat of broiler and its pathological alteration in liver and muscle tissue.

The ubiquitous presence of BHT, its controversial toxicological data, a lack of information about its true dietary intake, and also that of its metabolites have increased consumer concern about the use of this synthetic food additive, further research is needed to evaluate the current extent of human exposure to BHT and its metabolites.

Table (2): Effect of dietary natural (mushroom and vit. E&Se) and synthetic (BHT) antioxidants on weekly (Wk) live body weight (gm), body weight gain (gm/bird/week), feed intake (gm/bird/w), feed conversion ratio and performance index of broiler during experimental period.

Treatment / parameter	Gp.1	Gp.2	Gp.3	Gp.4	F-P value
Live bodyweight (gm)					
d 1	45	45	45	45	1.000
WK 1	195.00±2.00^{a}	195.00±2.00^{a}	195.00±2.00^{a}	195.00±2.00^{a}	1.000
WK 2	540.00±62.79^{a}	552.50±67.56^{a}	540.0±51.27^{a}	470.0±76.16^{b}	0.042*
WK 3	1030.67±91.30^{a}	1087.50±96.77^{b}	1020.0±74.07^{a}	844.44±231.47 ^{c}	0.003*
WK 4	1725.33±292.43^{a}	1833.75±162.65^{b}	1733.75±188.07^{a}	1506.25±293.59^{c}	0.043*
WK 5	2082.33±388.15^{a}	2289.38±172.39^{b}	2238.75±401.30^{b}	1708.2±275.31^{c}	0.004*
WK 6	2427.21±158.23^{a}	2739.38±152.26^{b}	26769.38±172.39^{b}	1868.52±145.31^{c}	0.004*
Body weight gain(g/bird)					
WK 1	150.00±2.00^{a}	150.00±2.00^{a}	150.00±2.00^{a}	150.00±2.00^{a}	1.000
WK 2	345.33±1.15^{a}	358.00±2.00^{b}	345.00±1.00^{a}	275.00±2.00^{b}	0.001*
WK 3	491.00±1.73^{a}	535.33±2.52^{b}	480.00±2.00 ^{c}	374.33±4.93 ^{d}	0.001*
WK 4	694.00±1.73^{a}	746.00±1.00^{b}	714.33±3.51^{c}	662.00±5.29 ^{d}	0.001*
WK 5	357.33±2.52^{a}	455.00±3.00^{b}	504.67±2.08 ^{c}	202.00±2.00 ^{d}	0.001*
WK 6	345.13±1.32^{a}	450.00±3.00^{b}	438.42±2.03 ^{c}	160.00±2.00 ^{d}	0.001*
Feed intake (gm/bird)					
WK 1	225.00±2.00^{a}	225.00±2.00^{a}	225.00±2.00^{a}	225.00±2.00^{a}	1.000
WK 2	453.00±2.65^{a}	440.00±4.00^{b}	488.00±1.00 ^{c}	422.00±2.00 ^{c}	0.001*
WK 3	753.00±2.00^{a}	1000.0±5.00^{b}	842.0±2.00 ^{c}	600.00±10.00 ^{d}	0.001*
WK 4	1030.0±3.00^{a}	1100.0±5.00^{b}	1045.33±2.51^{c}	914.00±2.00 ^{d}	0.001*
WK 5	1030.0±1.00^{a}	1086.0±1.00^{b}	1094.0±2.00 ^{c}	874.00±2.00 ^{d}	0.001*
WK 6	1000.0±1.00^{a}	1100.0±2.00^{b}	1090.0±2.00 ^{c}	763.00±2.00 ^{d}	0.001*
Feed conversion ratio					
WK 1	1.50±0.01^{a}	1.50±0.01^{a}	1.50±0.01^{a}	1.50±0.01^{a}	1.000
WK 2	1.31±0.01^{a}	1.23±0.01^{b}	1.41±0.01^{c}	1.53±0.006 ^{d}	0.001*
WK 3	1.53±0.01^{a}	1.87±0.01^{b}	1.75±0.01^{c}	1.60±0.01^{d}	0.001*
WK 4	1.48±0.01^{a}	1.47±0.01^{a}	1.46±0.01^{a}	1.38±0.01^{b}	0.001*
WK 5	2.88±0.02^{a}	2.39±0.01^{b}	2.17±0.01^{c}	4.33±0.02 ^{d}	0.001*
WK 6	2.89±0.02^{a}	2.44±0.01^{b}	2.48±0.01^{c}	4.70±0.02 ^{d}	0.001*
Performance index					
WK 1	13.00±1.00^{a}	13.00±1.00^{a}	13.00±1.00^{a}	13.00±1.00^{a}	1.000
WK 2	41.22±0.03^{a}	44.96±0.01^{b}	38.29±0.02^{c}	30.72±0.02 ^{d}	0.001*
WK 3	67.39±0.04^{a}	58.18±0.02^{b}	58.29±0.02^{c}	52.75±0.03 ^{d}	0.001*
WK 4	116.60±0.10^{a}	124.76±0.06^{b}	118.77±0.07^{c}	109.13±0.13 ^{d}	0.001*
WK 5	72.29±0.29^{a}	102.71±0.20^{b}	102.47±0.07^{b}	39.45±0.25 ^{c}	0.001*
WK 6	83.74±0.21^{a}	112.25±0.10^{b}	107.55±0.20^{b}	39.74±0.20 ^{c}	0.001*

Data were represented as means± SE. * Significantly difference using ANOVA test at p<0.05.

Table (3): Effect of dietary natural (mushroom and vit. E&Se) and synthetic (BHT) antioxidants on lipid profile of broiler chicken tissues.

Group / Organ	Gp 1	Gp 2	Gp 3	Gp 4	F- P value
Liver					
Triglyceride	**222.74±3.64^{a}**	**127.01±1.76^{b}**	**117.03±1.76^{c}**	**203.50±0.49^{d}**	**0.001***
Cholesterol	**97.44±0.02^{a}**	**76.48±0.34^{b}**	**56.72±1.42^{c}**	**132.30±0.53^{d}**	**0.001***
LDL	**63.51± 0.47^{a}**	**61.34±0.46^{b}**	**38.80±0.31^{c}**	**114.38±0.74^{d}**	**0.001***
HDL	**33.93±0.91^{a}**	**15.14±0.27^{b}**	**17.92±0.34^{c}**	**17.92±0.34^{c}**	**0.001***
Thigh					
Triglyceride	**251.60±0.61^{a}**	**202.00±1.88^{b}**	**137.74±1.72^{c}**	**218.00±2.64^{d}**	**0.001***
Cholesterol	**338.90±0.89^{a}**	**86.34±0.64^{b}**	**67.20±1.56^{c}**	**182.28±1.6^{d}**	**0.001***
LDL	**290.60±1.04^{a}**	**47.07±0.19^{b}**	**52.07±0.13^{c}**	**155.09±0.17^{d}**	**0.001***
HDL	**48.30±0.41^{a}**	**39.59±0.30^{b}**	**15.14±0.15^{c}**	**27.19±0.36^{d}**	**0.001***
Breast					
Triglyceride	**216.82±1.81^{a}**	**128.26±0.53^{b}**	**121.11±2.93^{c}**	**171.94±1.13^{d}**	**0.001***
Cholesterol	**258.50±0.52^{a}**	**65.46±1.14^{b}**	**57.12±3.27^{c}**	**134.22±0.86^{d}**	**0.001***
LDL	**200.40±0.50^{a}**	**56.81±0.90^{b}**	**47.23±0.60^{c}**	**117.04±0.43^{d}**	**0.001***
HDL	**58.10±0.26^{a}**	**8.65±0.48^{b}**	**9.89±0.16^{c}**	**17.18±0.29^{d}**	**0.001***

Data were represented as means± SE. * Significantly difference using ANOVA test at p<0.05.

Table (4): Residual level of BHT in raw and cooked meat of broiler chicken.

Treatment / Tissue	Raw	Cooked	Reduction percent	T-P value
Liver	**4.70±0.20**	**2.53±0.35**	**46.80**	**0.001***
Thigh	**3.17±0.25**	**1.43±0.25**	**54.74**	**0.001***
Breast	**1.83±0.31**	**0.10±0.17**	**94.53**	**0.001***

Data were represented as means± SE. * Significantly difference using T- test at p<0.05.

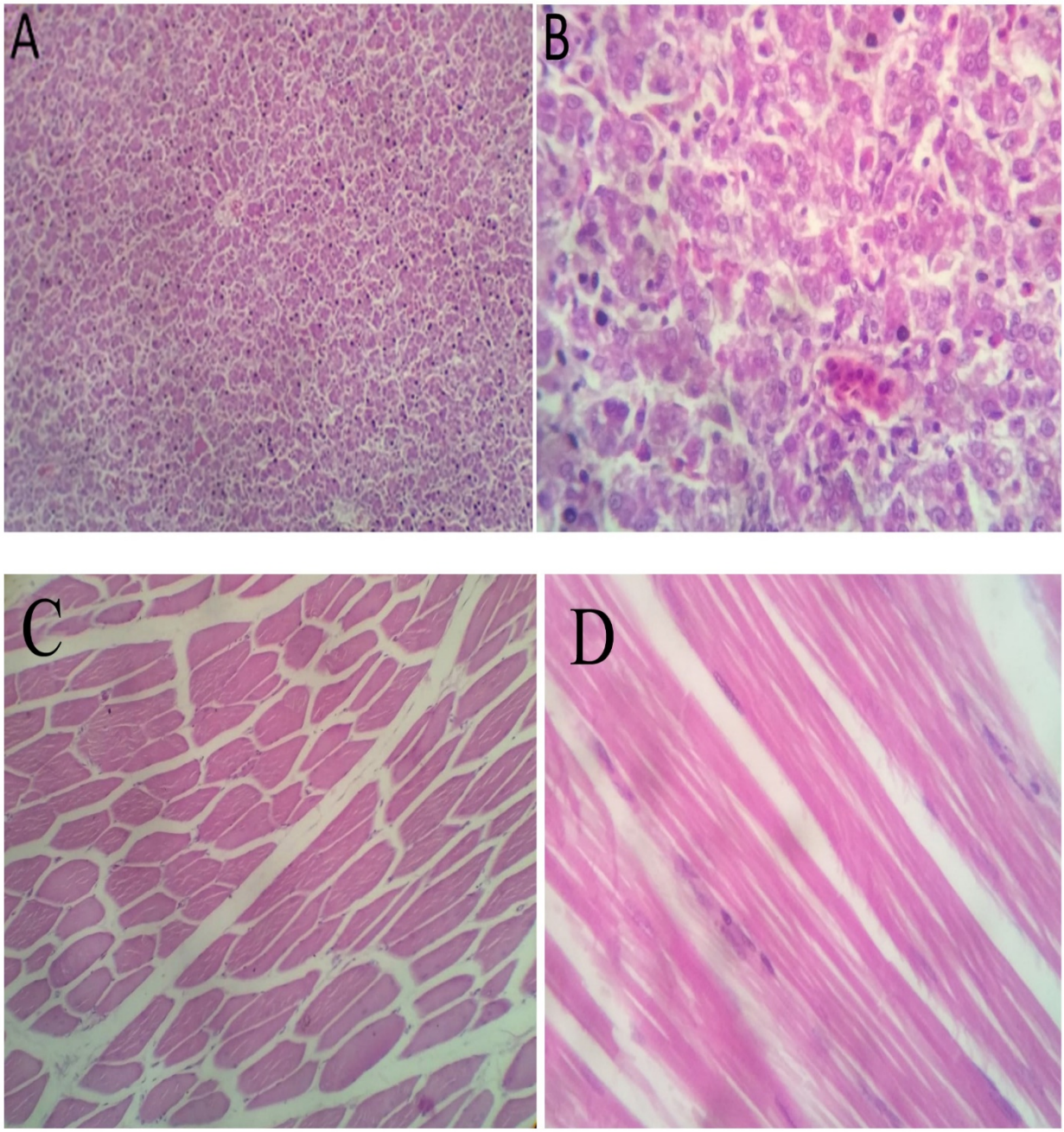

Fig. (1): (A, B, C and, D): Photomicrograph of liver Gp2 supplemented with dried mushroom showing nearly normal histomorphologic architecture **(A&B X100, 400).** Photomicrograph of skeletal muscle Gp2 showing apparently normal striations and peripherally located nuclei (C&D) **X400. H&E** stain.

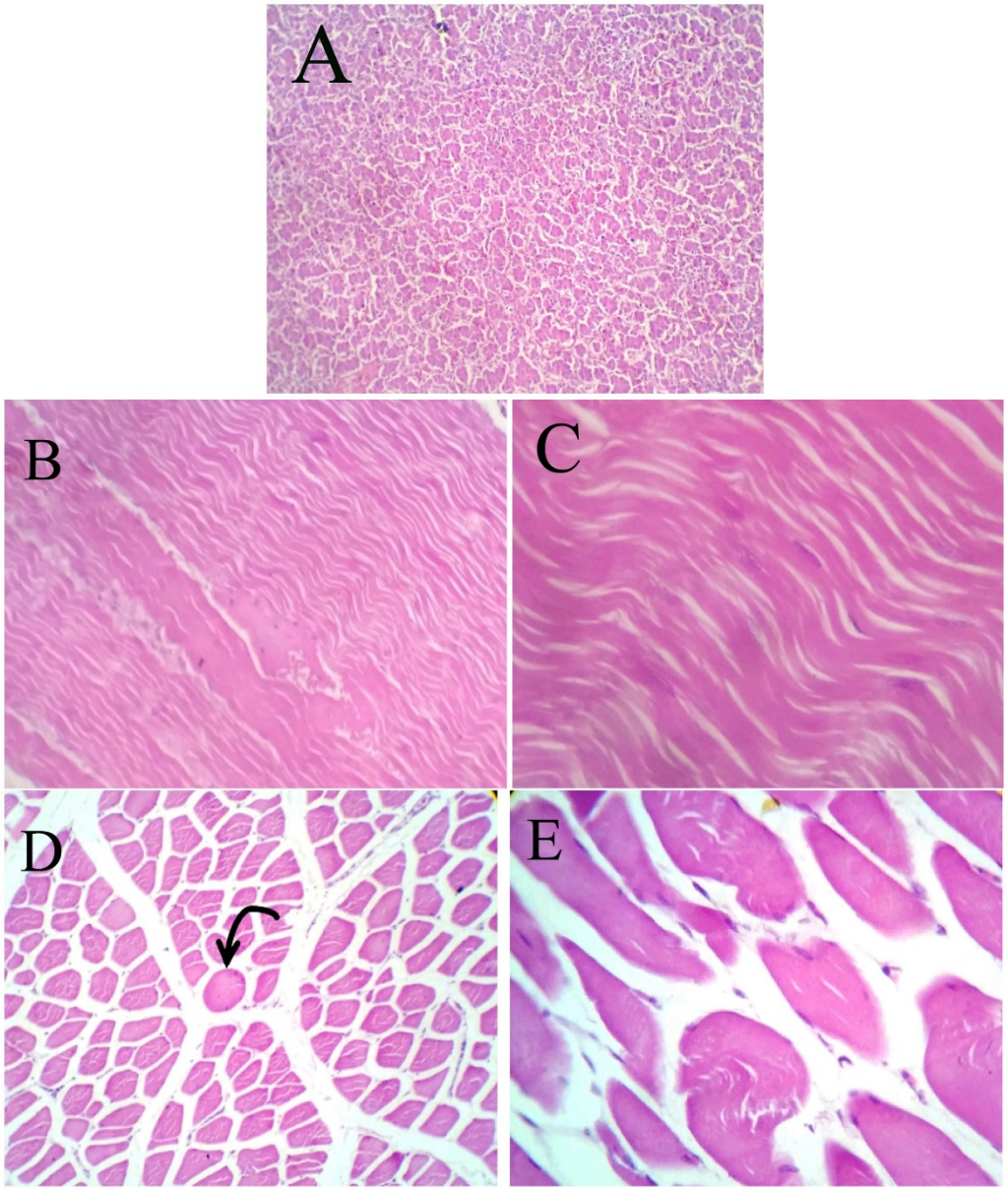

Fig. (2): (A, B, C, D and E) Photomicrograph of liver Gp3 supplemented with vit. E and Selenium showing nearly normal histomorphologic architecture **(A) X 100.** Photomicrograph of skeletal muscle of Gp3 showing preserved striation and interstitial tissue **(B&C) X.100&400,** a few fibers showing hyaline degeneration (curved arrow) with mild interstitial edema **(D&E) X 400. H&E.**

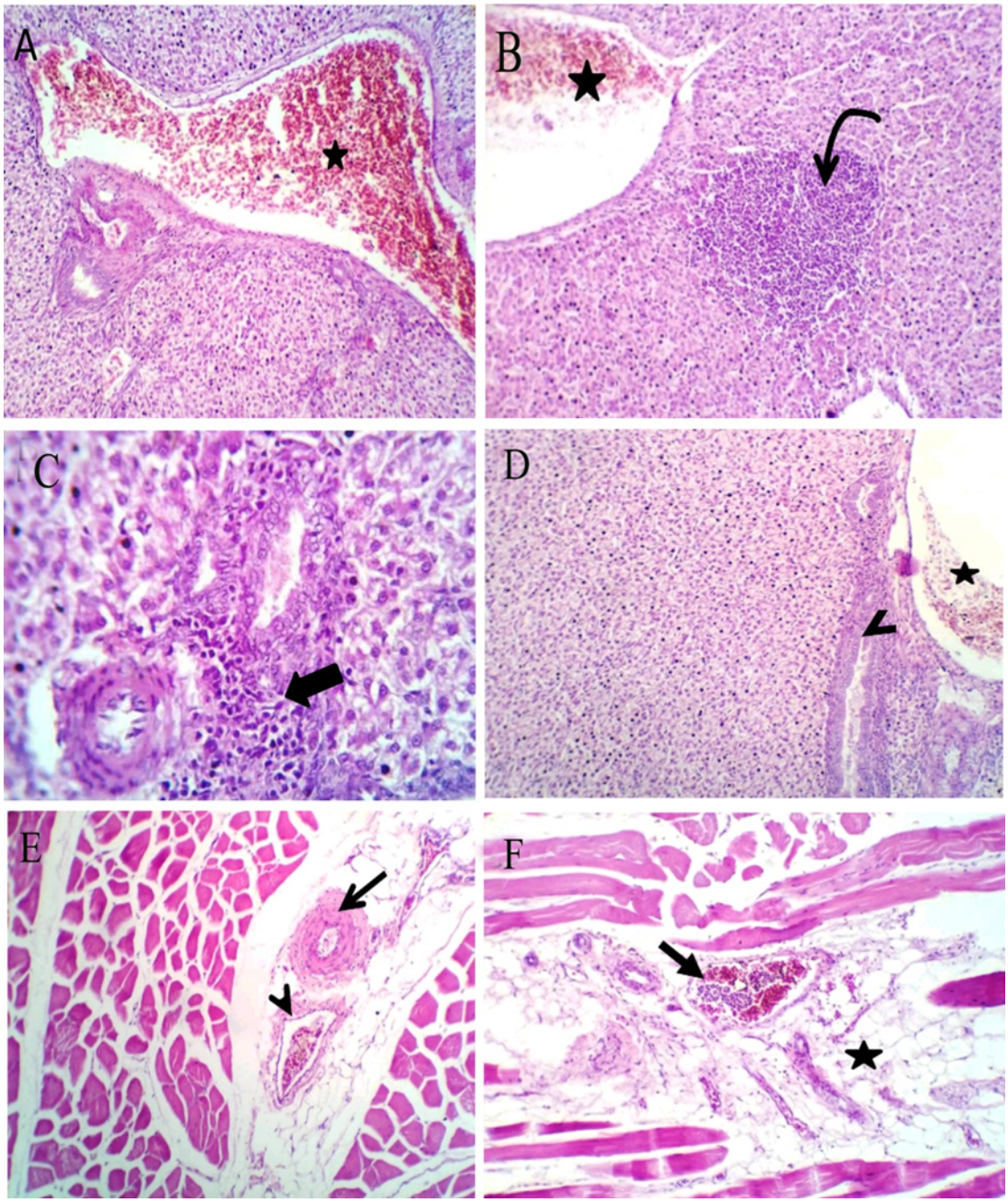

Fig. (3): (A, B, C, D, E and F) Photomicrograph of liver Gp.4 supplemented with BHT, showing congestion of hepatic blood vessels (star A), interstitial and portal round cells aggregation (curved B and thick arrow C), together with mild biliary proliferation (arrow head D) **(A&B&D X100), (C X400).** Photomicrograph of skeletal muscle of Gp4 showing swollen of the endothelium together with dilatation of the intermuscular arterioles (open arrow) and venules (arrow head), **(E, X. 100).** Congestion of muscular blood vessels (closed arrow) and lymphocytic infiltration together with perivascular edema (star), **(F, X. 100) .H&E stain.**

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تأثير مضادات الأكسدة الطبيعية والمخلقة كإضافات أعلاف على دجاج التسمين وصحة المستهلك

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الملخص العربى

أجريت هده الدراسة لمعرفة تأثير مضادات الأكسدة الطبيعية (المشروم الصالح للأكل وفيتامين هـ والسلينيوم) ومضادات الأكسدة الصناعية (بتيوليتد هيدروكسى تولوين BHT) كمكمل غذائى لدجاج التسمين على أداء النمو و الدهون فى الأنسجة والكشف عن متبقيات بتيوليتد هيدروكسى تولوين BHT فى اللحوم الغير ناضجة والمطهية (انسجة الكبد ؛الأوراك والصدور) فضلا عن التغيرات الباثولوجية لهده المواد المضادة للأكسدة على أنسجة كل من الكبد والعضلات. تم استخدام عدد 100 كتكوت عمر يوم وتم تقسيمها عشوئيا بعد عمر أسبوع الى اربع مجموعات. **المجموعة الأولى** تركت كضابط للتجربة تم تغذيتها على النظام الغذائى الأساسى (عليقة) المخصص لدجاج التسمين دون اضافة اى مكملات غذائية؛ **المجموعة الثانية** تم تغذيتها على النظام الغذائى الأساسى (عليقة) المخصص لدجاج التسمين مضافا اليها المشروم المجفف بجرعة تساوى 1.5جم/كجم عليقة؛ **المجموعة الثالثة** تم تغذيتها على النظام الغذائى الأساسى مضافا اليه فيتامين هـ والسلينيوم بجرعة تساوى 100مجم/كجم عليقة؛ **المجموعة الرابعة** تم تغذيتها على النظام الغذائى الأساسى (عليقة) مضافا اليها بتيوليتد هيدروكسى تولوين BHT بجرعة تساوى 100مجم/كجم عليقة. انتهت التجربة بعد 42 يوم حيث ذبحت الطيور وتم اخد نوعين من العينات من نسيج الكبد وعضلات الصدور والافخاد. تم حفظ العينات الاولى عند درجة حرارة -20درجة مئوية حتى يتم تطبيق القياسات المطلوبة . وتم تثبيت العينات الثانية فى محلول الفورمالين 10% لعمل الفحص الباثولوجى.

أظهرت النتائج التى تم الحصول عليها على زيادة معنوية فى أداء النمو فى مجموعات المشروم وفيتامين هـ والسيلسنيوم وانخفضت بشكل ملحوظ فى المجموعة التى تم اعطائها بتيوليتد هيدروكسى تولوين BHT.

كما لوحظ انخفاض ملحوظ فى دهون اللحم فى جميع المجموعات بينما لوحظت زيادة معنوية للكوليستيرول والبروتين الدهنى المنخفض الكثافة فى انسجة الكبد فى المجموعة التى تم اعطائها بتيوليتد هيدروكسى تولوين مقارنة بالمجموعة الضابطة للتجربة. كما تم الكشف عن متبقيات بتيوليتد هيدروكسى تولوين فى الكبد و اللحوم (اوراك وصدور) الغير مطهيه والمطهيه علاوه علي ذلك اظهرت النتائج علي ان الطهي عن طريق الغلي لمدة 30 دقيقة (السلق) ادت الي تخفيض بقايا بتيوليتد هيدروكسى تولوين في الكبد من 0.20 ± 4.70 الي 0.35 ± 2.53 بنسبة تناقص 46.80 كما كانت نسبة التناقص في الاوراك والصدور 54.74 و 94.53 علي التوالي .وقد اكدت النتائج ايضا ان نسبة المتبقيات في الاوراك اعلي من نسبتها في الصدور. لم يلاحظ تغيرات مرضية واضحة فى المجموعة التى تم اعطائها المشروم وفيتامين هـ والسيلنيوم بينما أظهر الفحص المجهرى للكبد احتقان فى الاوعية الدموية مع ارتشاح وفرط فى خلايا الدم المستديرة وتنكسات فى خلايا العضلات.

لدللك ، يمكن اضافة مضادات الاكسدة الى العلف لتفادى الاثار الضارة لاكسدة الدهون فى انتاج الدواجن حيث تؤثر على كل من الاعلاف التى تستهلكها الدواجن وكذلك الدواجن نفسها وبالتالى تؤثر الأكسدة على جودة وفترة صلاحية منتجات الدواجن للاستهلاك البشرى. ايضا يبدو ان المشروم وفيتامين هـ والسلينيوم حينما تستخدم كمكملات لغداء دجاج التسمين قد تكون افضل من بتيوليتد هيدروكسى تولوين BHT مضاد الاكسدة الصناعى.

هذا وقد تمت مناقشه الاهميه الصحيه لمضادات الاكسده الطبيعيه والصناعيه كمكملات اعلاف دجاج التسمين ومدي تاثيرها علي جوده اللحوم و بالتالي صحه المستهلك .