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EVALUATION OF THE EFFECT OF USING GRAPE SEED OIL (*VITIS VINFERA L.*) AND BLACK CUMIN SEED OIL (*NIGELLA SATIVA*) IN BROILER DIET IN SOME BLOOD PHYSIOLOGICAL TRAITS AND OXIDANT STATUS IN MEAT

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Abstract

This study was conducted at the Poultry Researches Station / Department of Animal Resource/Directorate of Agricultural Researches/Ministry of Agriculture, during the period from 20/5/2018 to 20/7/2018 to study the effect of adding grape seed oil and black cumin seed oil in broiler diet on some physiological trait and oxidant state in meat.

In this study 300 (Ross 308) broilers at age of 21 days has been used, these birds had randomly distributed to 5 dietary treatments, each treatment has three replicated (20 birds/ replicate) T1 control treatment without adding grape seed oil or black cumin while T2 was used 1 % of grape seed oil, T3 used 2% grape seed oil, wherever 1% of black cumin oil used in T4, and 2% black cumin oil used in the T5. The birds had been fed with one diet during the experimental period and the diets content was calculated as (NRC, 1994), the results of this study showed:

There is a high significant difference (P<0.01) in total plasma protein and globulin in T3 and T5 compared with T1, and a high significant decreased (P<0.01) in cholesterol levels in serum for T2,T3, T4 and T5 were compared it with T1 treatment, and there is a high significant decrease (P<0.01) in same trait for T5 compared to T2, T4 treatment, in the same time the results showed a high significant increased (P<0.01) in high density lipoprotein (HDL)for the additive treatments (T2,T3,T4 and T5) compared to control T1, and there is a high significant decreased (P<0.01) in low density lipoprotein (LDL) in treatments T2,T3,T4 and T5 compared to T1 treatment. For the triglyceride the results showed a high significant decreased (P<0.01) in blood plasma for T2 treatment compared with other treatment, and there is a high significant difference (P<0.01) in Malondialdehyde in meat for the T2,T3 and T5 treatments compared with control treatment and peroxide value in meat was high significantly decreased (P<0.01) in additive treatments compared with control treatment, and also there is high significant decreased (P<0.01) in free fatty acids levels in meat for T2 and T3 treatments compared with other treatments. The results showed a highly significant decrease (P<0.01) in peroxide value in meat for the T2, T3 treatment compared to T1, T4 and T5, also there is a significant decrease (P<0.01) in same trait for T5 treatment compared to T1 treatment.

***Keywords*:** Grape seed oil, Black cumin seed oil, Natural antioxidant, physiological traits

Introduction

Over the past few decades, there has been increasing interest by researchers on the efficacy of therapeutic oils for the purpose of improving the productive and physiological performance of animals, including domestic poultry, and their use as an alternative to preservatives for the production of healthy and functional foods (Elgayyar *et al*., 2001), for avoiding the negative effects of the use of medicinal drugs with the chemical origin of the birds, and to maintain consumer health and enhance the immunity of the body by stimulating the immune system (Azeem *et al*., 2014).

Therapeutic oils are the latest sources used in poultry feed, such as broilers because it contain effective contains which in turn improve the reproductive and productive performance of birds (Jamroz and Kamel, 2002).

Grape (*Vitis vinfera L.*) is one of the most produced fruit in the world, grape seed oil obtained from the seed of grapes (Wren *et al*., 2002) compared to other oil seeds, grape seed oil is rich in term of unsaturated fatty acids such as oleic and linoleic acid (Barron *et al*., 1988)The unsaturated fatty acids like oleic and linoleic acid are essential for the human metabolism (Baydar and Akkurt, 2001), grape seed extract (aqueous or alcoholic) has high antioxidant potential, its beneficial effects include the modulation of antioxidant enzyme expression (Baydar and Akkurt, 2001), production against oxidative damage in cells, anti-atherosclerotic and anti-inflammatory effect and protection against some cancer types in both human and animal models (Anonymous, 2007 ; Bloom, 2009). Grape seed oil contains a large amount of phenolic compound, including flavonoids, carotenoids, phenolic acid, tannins and silences and Gallic acid, which have been reported to be in a wide range of biological activities but are mostly known for their antioxidant properties (Xia *et al*., 2010), some researchers have stated that grape seed oil helps vitamin C to enter the body's cells, that strengthening cell membranes and protecting cells from oxidative damage of free radicals (Veria Living, 2012).

And other researcher used a Black cumin seed oil as therapeutic oils because it is one of the oils with a high content of polyunsaturated fatty acids as well as its high content of omega compounds and its containment of natural antioxidants, especially vitamin E, which plays an essential role as an antioxidant as it works to protect the cell from free radicals (Mahbubur and Shang, 2016), one of the benefits of high efficient black cumin seed oil is to eliminate some pathogenic microorganisms that may have resistance to many antibiotics and medical drugs (Azeem *et al*., 2014). Black cumin seed oil is a high-fat polyunsaturated fatty acid, in addition to its high content of omega compounds in general and containing natural antioxidants, especially vitamin E, whose role is mainly antioxidant, which works to protect the cell from free radicals (Mahbubur and Shang, 2016).

The aim of this study was to investigate the effect of adding different levels of grape seed oil and black cumin seed oil to broiler and its effect on the and physiological performance and the state of oxidation of meat and its content of fatty acids.

Materials and Methods

Birds and Dietary treatment:

This study was carried out at the poultry research/ office of the agricultural research / ministry of agriculture for the period 20/5/2018 to 20/7/2018. 300 (Ross 308) broilers at age of 21 days has been used and randomly distributed to five dietary treatments with three replicated (20 birds/ replicates), T1 control treatment without additive, T2 adds 1% grape seed oil , T3 adds 2% grape seed oil, T4 adds 1% black cumin seed oil and T5 add 2% black cumin seed oil. All the treatments gave the same diet in all the experiment period which showed in table (1).

Table 1: Percentage composition of the experimental diets

Ingredients %	Types of diets		
	Starter 1-13D.	Grower 14-27d.	Finisher 28-42d.
Wheat	60.32	62.93	69.9
Soybean Meal [1]	29	25.7	18
Meat Meal [2]	5	5	5
Hydrogenated Vegetable Fat	3.4	4.5	5.4
Dicalcium Phosphate	1.1	0.8	0.7
NaCl	0.1	0.1	0.1
Limestone	0.7	0.7	0.6
Methionine	0.17	0.17	0.15
Lysine	0.21	0.10	0.15
Total	100	100	100
Calculated Values[3]			
M.E. Kcal/ Kg Diet	3001	3101	3211
Crude Protein %	**23.5**	22.2	19.4
Crude Fat %	5.4	6.6	7.6
Crude Fiber %	**3.1**	3	2.9
Lysine, %	**1.4**	1.23	.06
Methionine Plus Cysteine %	**1.00**	0.97	0.86
Ca , %	0.89	0.81	0.73
Available P, %	2.82	2.86	3.10

[1]Soybean cake used an Argentine source of crude protein content by 48% and 2440 Kcal/ Kg M.E.
[2] Protein Meal User Product From Netherlands Origin **(**Brocon**)** Contain 40% Crude Protein 0.2107 Kcal / Kg Protein M.E., 0.5% Crude Fat 2.20% Crude Fiber 5%, Calcium 4.68% ,Phosphorus 3.85% Lysine 4.12%, Methionine 4.12% , Methionine Plus Cysteine 0.42%, Tryptophan 0.38%, Threonine 1.70%. It Contains A Mixture Of Vitamins And Minerals Needed Believes Rare Birds Of These Elements.
[3]Based on (NRC, 1994).

Blood samples and analysis

At the termination of the study, at 42 days of age, 9 birds per group totaling 3 birds per treatment replication, was removed randomly for blood collection. Blood samples (1 ml/bird) were collected from the ulnaris wing vein into EDTA tubes. Within two hours after blood samples were collected, they were centrifuged (3000 X *g*, for 10 min at room temperature) to separate plasma from blood cells, and plasma was then decanted and stored in Eppendorf safe-lock micro-centrifuge tubes at –20 °C until assayed. Blood parameters analyzed in this study were: total protein, albumin, globulin, cholesterol, triglycerides; very low density lipoprotein (VLDL), high density lipoprotein (HDL), low density lipoprotein (LDL).

-Gas chromatographic analysis of fatty acid profile of meat:

Meat samples were collected and carried out in the general company for vegetable oil, ministry of industry-Iraq, for the fatty acid profile analysis the collected meat sample carried out with the help of GC device (SHIMADZU Model 17-Japan) (A.O.A.C., 2005).

Statistical Analysis:

Completely randomized design (CRD) was used to study the effect of different treatment in all traits, (Duncan, 1955) and multiple range tests was used to compare the significant differences between means. Data were analyzed by using statistical analysis system (SAS, 2012).

Results and Discussion

Table (2) showed that there is a high significant increase ($P<0.01$) in total plasma protein in T3, T5 treatments, similar result were founded by (16) that added 2% black cumin seed oil to diet led to a highly significant increase ($P<0.01$) in total plasma protein, and also this result come agreement with (AL-Hothaify and Al-Sanabani, 2016) which he observed increase in total plasma protein in a 1% black cumin seed oil treatment.

The results from table (2) showed also that there is no significant differences in albumin in all treatments, and this result similar to (Al-Jaff, 2014) when found that there is no significant differences in albumin levels in layer hen when adding a different levels of grape seed oil, and this result was not similar to AL-Hothaify and Al-Sanabani, 2016) that he

observed a high significant increase (P<0.01) in albumin when added 1% of black cumin seed oil to the diet.

Also can observe from the table (2) a high significant increase (P<0.01) in globulin level in T3, T5 treatment, while there was a high different significant (P<0.01) in T4 compared to T1, T2, this results not agreement with (AL-Hothaify and Al-Sanabani, 2016).

This may be attributed to enzymes from black cumin seed oil which led to increase enzyme synthesis in liver (Al-Jishi and Abuo Hozaifa, 2003).

Table 2: Effect of using grape seed oil and black cumin seed oil in broiler diet on total protein, albumin and globulin levels in blood plasma

Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
T1	2.94 ± 0.12 b	1.65 ± 0.33	1.26 ± 0.12 c
T2	2.97 ± 0.07 b	1.74 ± 0.26	1.23 ± 0.08 c
T3	3.52 ± 0.91 a	1.30 ± 0.27	2.22 ± 0.09 a
T4	3.21 ± 0.19 ab	1.42 ± 0.34	1.79 ± 0.17 b
T5	3.67 ± 0.10 a	1.34 ± 0.40	2.33 ± 0.09 a
Significantly	**	N.S	**

The means with different letters within the same column are significantly between them (P<0.01) **, NS= No significant

Observed from table (3) a highly significant decrease (P<0.01) in cholesterol levels in plasma for T2, T3, T4 and T5 compared to T1, and there is a highly significant decrease (P<0.01) in cholesterol for T5 compared to T2, T4 treatment, and at the same time there is a high significant increase (P<0.01) in high density lipoprotein for all adding treatment compared to control treatment, and a highly significant decrease (P<0.01) in Low density lipoprotein levels for all adding treatment compared to control treatment, while there in no significant differences in very low density lipoprotein levels for all treatment, this results agreement with (Jaffer, 2017) that he observed a highly significant decrease (P<0.01) in cholesterol levels, and agreement with (Tekeli *et al*., 2014) when he observed no significant differences in very low density lipoprotein levels when he added grape seed oil 0, 5, 10, 15 g/ kg diet for broiler, and this may be due to Grape seed oil has an abundance of antioxidant substances that lower cholesterol level, and it contains lineolic acid that acts to increase high density lipoprotein (HDL) or decrease cholesterol level and low density lipoprotein (LDL) level (Park et al. 2016). This may be due to the biological mechanism underlying the antioxidant property is associated with the removal of free radicals, mainly hydroxyl radical, and chelation of metals, which influence cell signaling and functioning of the immune system, this is of particular importance when considering the capacity of grape seed extract to attenuate oxidative stress and decrease low-density lipoprotein (LDL) levels, and thereby reduce the inflammatory process related to some diseases. and this results similar to (AL-Beitawi and EL-Ghousein, 2008) that he observed a significant decrease (P<0.05) in cholesterol level and increase in high density lipoprotein level in blood plasma due to used black cumin seed oil, and agreement with (Hermes et al. 2011) that he found a significant decrease (P<0.05) in cholesterol level in blood plasma when added 0.5 % Nigella sativa seed oil and 1% Nigella sativa and 10% Nigella sativa meal to broiler diet, also this results similar to (AL-Hothaify and Al-Sanabani, 2016) when he used a different levels from black cumin seed a significant decrease (P<0.05) in cholesterol level in blood plasma, and this results also agreement to (Akhtar *et al*., 2003) that he found a significant decrease (P<0.05) in cholesterol and low density lipoprotein levels and a significant increase (P<0.05) in high density lipoprotein level in blood plasma when he used 0.5, 1, 1.5 % Nigella sativa powder in layer hen diet, these results may be due to black seeds have been reported to possess a favorable effect on serum lipid profile by decreasing it total cholesterol, low density lipoprotein and triglyceride by elevating the high density lipoprotein level (El-Dakhakheny *et al*., 2000).

Table 3: Effect of using grape seed oil and black cumin seed oil in broiler diet on cholesterol, HDL, LDL and VLDL levels in blood plasma

Treatment	**Cholesterol (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
T1	176.42 ± 7.20 a	64.08 ± 0.20 b	80.678 ± 0.30 a	31.662 ± 0.01
T2	147.61 ± 10.70 b	84.21 ± 0.16 a	34.766 ± 0.18 b	28.634 ± 0.03
T3	131.73 ± 8.78 bc	78.71 ± 0.22 a	22.496 ± 0.35 b	30.524 ± 0.06
T4	140.33 ± 8.30 b	88.04 ± 0.20 a	22.690 ± 0.39b	29.600 ± 0.08
T5	128.25 ± 9.31 c	78.13 ± 0.31a	20.896 ± 0.25 b	29.224 ± 0.03
Significantly	**	**	**	N.S

The means with different letters within the same column are significantly between them (P<0.01) **, NS= No significant
HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein

Figure (1) showed that a significant decrease (P<0.05) in triglyceride in blood plasma for T2 compared to T1 treatment, this results was similar to (Jaffer, 2017) there is a significant decrease (P<0.05) in triglyceride level in plasma when used at a different levels of grape seed oil in broiler diet, and this results come due to that grape seed oil effect on fat metabolism and that led to decrease triglyceride in serum and liver (Ozgan *et al*., 2009) and these results also agreement with (Nakamura and Tonogai, 2002) that he noticed that grape seed oil effect on fat metabolism therefore decrease triglyceride level in liver.

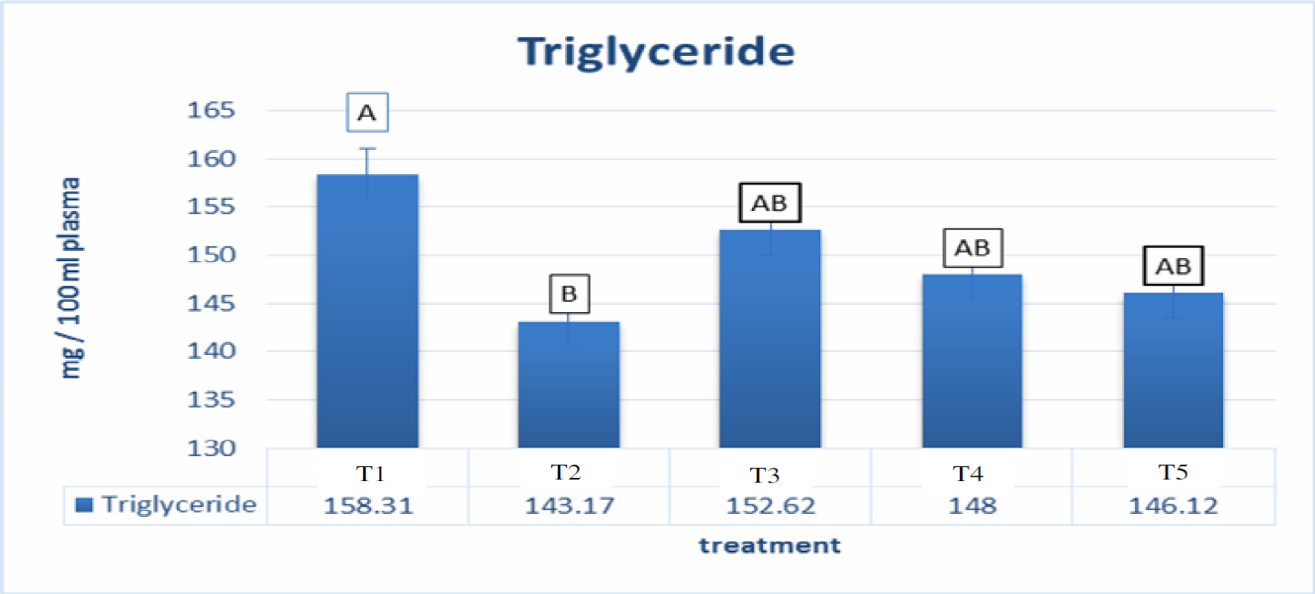

Fig. 1 : Effect of using grape seed oil and black cumin seed oil in broiler diet on triglyceride levels in blood plasma

The results from figure (2) a significant lower (P<0.05) in malondialdehyde level in meat for T2, T3 an T5 compared to T1 treatment, and also T3 decreased when compared it with T2, T4 and T5 in malondialdehyde level in meat, this result similar to (Al-Jaff, 2014) that found a significant improvement (P<0.05) in malondialdehyde level in the liver for grape seed oil treatment compared to control treatment, it considers malondialdehyde one of the second production of the oxidation process and peroxide crashes, it's also a guide to measure fat oxidation in blood plasma and tissue because it is more stable than hydro peroxides and peroxide (Pokorny *et al*., 2001), this result is similar to (Tuluce *et al*., 2009) that observed a significant decrease (P<0.05) in malondialdehyde level when used 1% black cumin seed compared to control treatment and this happened due to black cumin caused protective effects on the oxidative stress by inhibiting free radical production, and may be due to that Diet treated with 0.5 and 1% black seeds resulted in significantly decreased erythrocyte malondialdehyde (MDA) concentration, production of lipid peroxides. The scientist concluded that *Nigella sativa* exhibits protective properties on the injury produced by oxidative stress by inhibiting free radical production and by regulation of glutathione preventing oxidative stress. Black seeds might decrease the production of hydrogen peroxide (H_2O_2), hydroxyl (OH) and superoxide (O_2) radicals that are produced as a result of aerobic respiration (Tuluce *et al*., 2009).

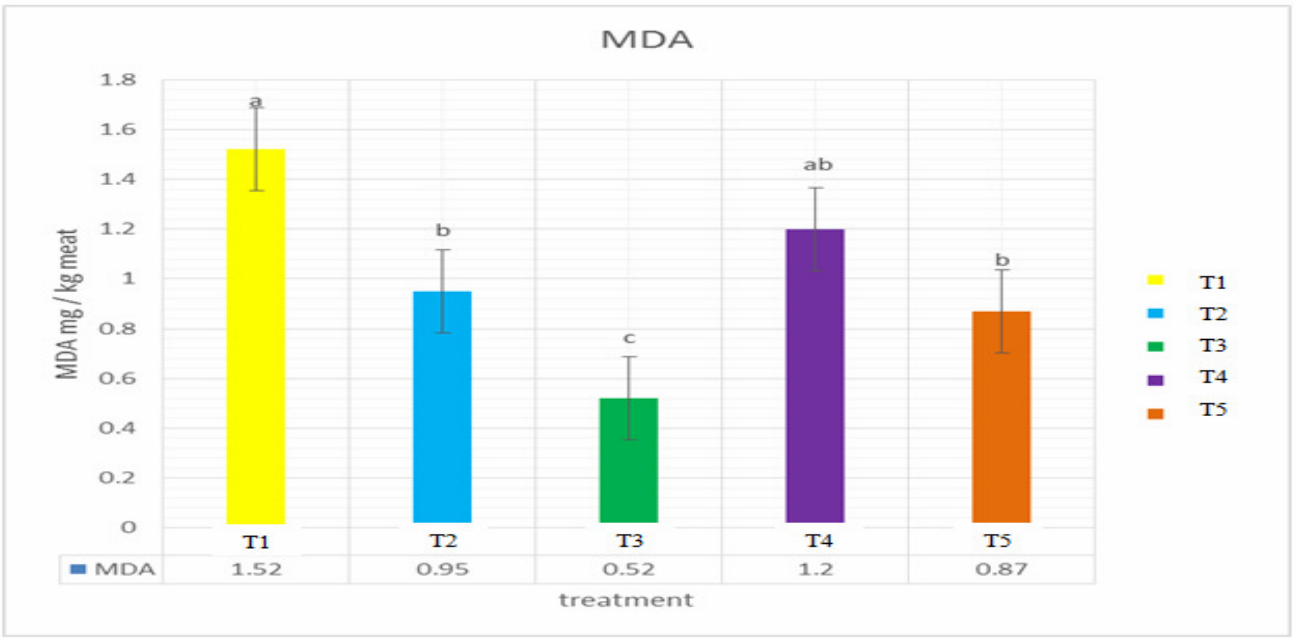

Fig. 2 : Effect of using grape seed oil and black cumin seed oil in broiler diet on t malondialdehyde levels in meat

Observed from Figure (3) a significant decrease (P<0.05) in free fatty acid levels in meat for the T2, T3 compared to other treatment, and this results is agreement with (Al-Jaff, 2014) when noticed a significant decrease (P<0.05) in free fatty acid for grape seed oil, this may due to that antioxidants provide protection for lipid membranes by interacting with free radicals and breaking the chain of fat oxidation reactions and thus restricting the release of free fatty acids (Akarpat *et al*., 2008).

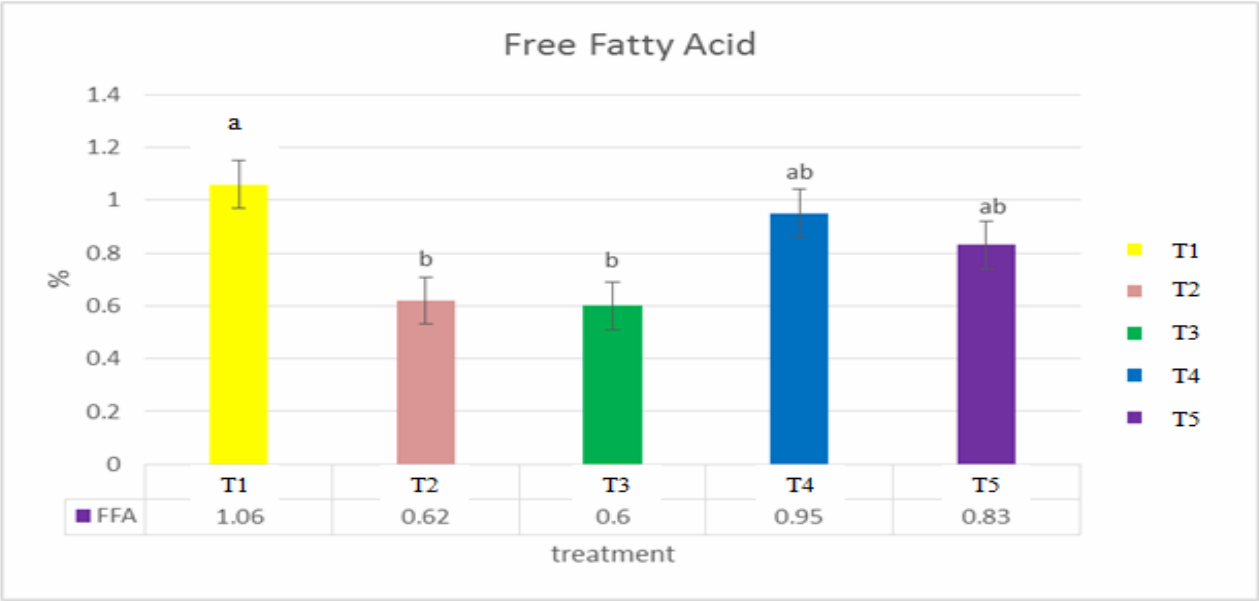

Fig. 3 : Effect of using grape seed oil and black cumin seed oil in broiler diet on free fatty acid levels in blood plasma

The results from figure (4)showed a highly significant decrease (P<0.01) in peroxide value in meat for the T2, T3 treatment compared to T1, T4 and T5, also there is a significant decrease (P<0.01) in same trait for T5 treatment compared to T1 treatment, and this may be due to vitamin E contributes to the beneficial effects of grape seed oil, because of its high antioxidant activity (Shinagawa et al. 2015), and may be due to the most notable bioactive property of phenolic compounds is their antioxidative capacity. This property has been widely studied in grape seed extracts which compounds are capable of scavenging ROS and inhibiting lipid oxidation (Xia *et al*., 2010), This high antioxidant capacity is related to the high content of Gallic acid, catechin, epicatechin, procyanidins, and proanthocyanidins in grape seed and seed oil (Hernández-Jiménez *et al*., 2009) and may be a result of the synergistic combination of these phenolic compounds (Khurana *et al*., 2013). And may be due to The study carried out on broilers showed that the *N. sativa* decreased the hepatic liver peroxidation and increased the activities of several enzymes such as glutathione-S-transferase, catalase, myeloperoxidase and adenosine deaminase all of which resulted in decreased oxidative stress on the liver using 3, 5 and 7% black cumin (Sogut *et al*., 2008), and may be due to black seed oil reduced the lipid peroxidation activities of liver enzymes and contributed to the antioxidant defense system (Kanter *et al*., 2003).

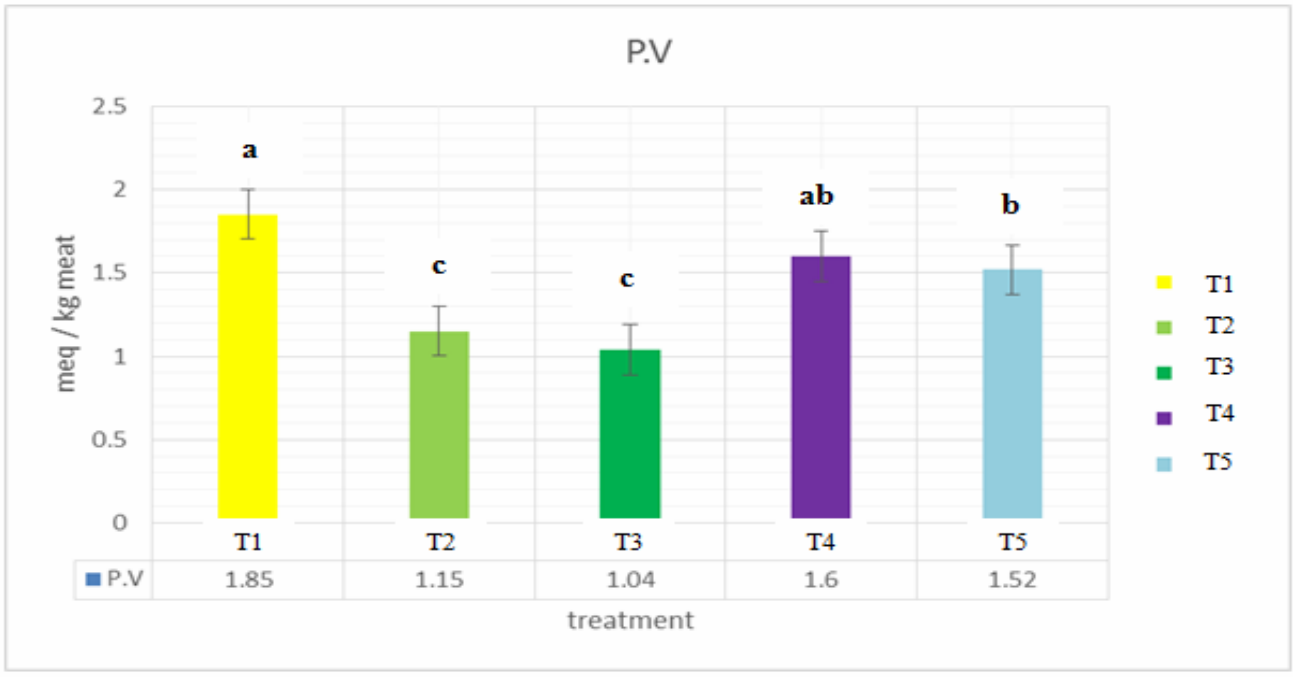

Fig. 4 : Effect of using grape seed oil and black cumin seed oil in broiler diet on peroxide value in meat

Conclusion

From the results of this study we conclude that use 2 % grape seed oil in total protein and triglyceride, while, 1,2% grape seed oil and 1,2 % black cumin treatments distinction for cholesterol, HDL and LDL traits, 1, 2% grape seed oil and 2 % black cumin distinction for malondialdehyde in meat, peroxide value in meat was decreased in additive treatments, also there is decreased in free fatty acids levels and peroxide value in meat in meat for 1,2 % grape seed oil treatments.

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