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EFFECTS OF CALCIUM SALT OF FATTY ACIDS ON GROWTH PERFORMANCE AND BIOCHEMICAL PARAMETERS IN BROILER CHICKENS

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ABSTRACT

The aim of this study is to determine if calcium salts of fatty acids (CSFA) can replace soybean oil in broilers diet. So 60 sex mixed Cobb chicks 10 day old divided into 3 groups, each included 20 chicks, and subdivided into 2 subgroups each included 10 chicks. All fed the same starter diet up to 10 days of age then fed grower and finisher diet with different dietary fat sources. Groups were: G_1: basal diet with CSFA (Poly fat®) (PF), G_2: basal diet with soybean oil (SO), G_3: basal diet with Poly fat® + soybean oil 50:50 (PF:SO) at grower period, and 75:25 (PF:SO) at finisher period.

Adding CSFA alone to broilers diet lead to a non significant decrease in the live body weight and feed conversion ratio (FCR)= 2, G_3 had the best FCR=1.8 with a non significant increase in live body weight. CSFA showed a significant decrease in malondialdehyde (MDA) ($P \leq 0.05$), a non significant decrease in the level of blood total cholesterol, HDL Cholesterol and LDL Cholesterol, this was supported by gene expression of cholesterol carrier protein that showed a decrease in its expression in G_1 and G_3 when compared with control group but there was a significant decrease in blood triacylglycerol in G_1 when compared with G_3 ($P \leq 0.05$) that showed the lowest results in blood total cholesterol, there was a significant increase in reduced glutathione (GSH) content in blood ($P \leq 0.001$) and liver tissue ($P \leq 0.05$) and a non significant increase in glutathione peroxidase activity in both blood and liver tissue in PF group when compared with control group, adding CSFA had no effect on both blood calcium and inorganic phosphorus.

Conclusion: *CSFA helps in reducing lipid peroxidation, oxidative stress and cholesterol level in broiler chicks.*

Keywords: *[Calcium salts of fatty acids; dry fat; lipid peroxidation; antioxidants; cholesterol }*

INTRODUCTION

Lipids constitute the main energy reserve of animals and it has the highest caloric value among all nutrients **(Lehninger et al., 2000)**. Moreover lipids are too important not only to supply birds with essential fatty acids but also to improve the digestibility of other non fat components **(Baião and Lara 2005)**.

Efforts have been made to substitute animal fat in feeding broilers with vegetable oil. Unlike animal fat, vegetable oil is more preferable as they contain higher level of poly unsaturated fatty acids, have a positive effect on performance specially sunflower oil **(Bilal et al., 2001)**. The inclusion of oil in broiler diets increases dietary energy density, improves feed palatability, reduces feed dust,

and improves feed conversion ratio and weight gain **(Baião and Lara 2005)**. The main problem for using vegetable oil in poultry diets in developing countries is mixing oil with diet because there is no unsuitable facilities for such a purpose. Today vegetable oil are used in the form of dry fat instead or mixed with oily sources. As this dry fat specially calcium salts of fatty acids offer some advantages as: higher stability, suitable application form (solid loose substances), and represents an important source for calcium **(Malá et al., 2004)**.

Researchers showed a non significant differences between chickens fed soy bean oil and experimental groups of calcium salts of fatty acids **(Malá et al., 2004 & Tabeidian and Sadeghi 2006)**, while **Nima Mosavat (2011)** found that the use of soybean fatty acids as compared with soybean calcium salt fatty acids had a helpful effects on broiler performance.

The type of dietary fat can affect the plasma GPx levels in chicks, **Halamíčková et al**., **(2006) & Marja et al., (1984)** also reported that the content of malondialdehyde in the thigh muscles was significantly lower in chickens fed soybean oil than in chickens fed Ca-salts of rapeseed and fatty acid of soybean oil.

Mutassim (2013) detected a significant higher concentration of blood serum cholesterol was found in chicks fed dry fat but the inclusion of dry fat with yeast culture positively affected the carcass characteristics by reducing the abdominal fat and blood cholesterol level. It was reported that no significant differences were observed in plasma triacylglycerol, cholesterol, HDL, LDL, serum Ca and inorganic P when calcium salts of fatty acid included in chicken diet **(Malá et al., 2004 and Tabeidian & Sadeghi 2006)**. Glutathione peroxidase GPx is a major selenium containing enzymes found in mammalian cells **(Stanley et al., 1974)**. The apparent selenium absorption was maximum when dietary calcium was 0.8% of dry matter intake. Amounts of dietary calcium less or greater than 0.8% of dry matter intake reduced apparent selenium absorption, while other authors reported that selenium status did not differ with calcium supplementation in human **(Harrison et al., 1984 & Holben et al., 2002).**

MATERIAL AND METHODS

1) Experimental birds:

60 sex mixed 10 day old Cobb broiler chicks weighed 230-260 gm., divided equally with respect in to three equal groups, each group included 20 chick subdivided into 2 equal subgroups(10 per pen). All pens (experimental unit) were bedded with a wheat straw litter and equipped with feed and water utensils after a very well disinfection of the pens and utensils. All chicks are reared under the same environmental conditions, the same managerial procedures and under temperature of 35-36 c then gradual decreasing till reach 20-24 at the end of the experiment. Alight/dark cycle of 23/1 h. The experiment was conducted following the guidelines of the Institutional Animal Ethics Committee. The experiment was carried out on December and January and last for 4 weeks.

2) Experimental diet:

Birds were given ad libitum access to water and the diet, fed the same starter diet up to 10 days of age, then switched to two phased diets (grower ; 11- 22 days of age, then finisher; 23-42 days of age) according to guidelines of cobb requirements as shown in table (1).

The diet contain different dietary fat sources (poly fat®, soybean oil, mix between them), To provide iso-caloric diets, poly

fat(PF) was included at the rate of 3% at grower period and 4% at finisher period.. First group (G_1).

Soybean oil (SO) at the rate of 3.5% (grower period) and 3.2% at finisher period.. Second group (G_2),as control group

PF+ SO at the rate of 1.7% + 1.5%(50:50 at grower period) and 2.6%+ 0.95% (75:25 at finisher period) third group (G_3)

Poly fat was provided by Norel-Misr, Egypt , and according to the manufacture it is calcium salts of 70% palm oil fatty acids, 25% sunflower and corn oils and 5% soybean oil, 7000 Kcal/Kg, with 0.35% an acid value and 3.0 mM O_2/kg peroxide number. The fatty acids pattern of Polyfat® comprises 57.6% unsaturated fatty acids and 42.4% saturated fatty acids, while calcium contributes with 8.55% of the product.

Chicks were weighed at day 22 and 42 of age. Birds in each group were closely monitored, during the whole period of the fattening the chickens showed no clinical symptoms of diseases, no mortalities were recorded.

Sampling:

1) Blood samples:

At the end of the experiment 14 blood samples per group (7/replicate) were obtained by slaughtering after 12 h fasting period (water supplementation were available till slaughtering). Blood samples were collected in sterile, clean and dry screw capped centrifugal tubes.

7-10 ml of blood sample per chick were divided into two parts:

- The first part was collected in dry tubes without anticoagulant and left for clotting at room temperature, then centrifuged at 3000 r.p.m for 15 minutes for serum separation, the collected serum samples were used for determination of lipid peroxide (malondialdehyde) **(Satoh 1978)**, total cholesterol **(Allain et al.,1974)**, HDL-Cholesterol **(Burstein et al., 1970)**, LDL-Cholesterol **(Wieland and Seidel.,1983)**, triacylglycerol **(Fossati et al., 1982)**, calcium **(Gindler and King 1972)** and inorganic phosphorus **(El-Merzabani et al., 1977)** .
- Second part of blood was collected in tubes with anticoagulant that was used for determination of glutathione peroxidase activity **(Paglia and Valentine 1967)** and Analysis of non-enzymatic antioxidant (reduced glutathione) **(Beutler 1963)**, both in erythrocyte lysate. Samples stored at −70C To minimize GSH auto oxidation and rapid enzymatic proteolysis, and processed under refrigeration **(Camera and Picardo 2002)**.

2) Dissection of birds:

After the end of blood collection procedures,10 chickens per group were dissected to obtain liver tissue. Organs were washed by normal saline and used for determination of tissue reduced glutathione content **(Beutler et al., 1963)** and tissue glutathione peroxidase activity **(Paglia and Valentine 1967)**.

3) Real time polymerase chain reaction (RT-PCR) samples:

Intestinal specimens of chicken were taken as 3 samples per group and washed by normal saline then preserved directly in RNA liter for quantitative PCR for detection the effect on gene expression.

Primer to the gene of Niemann-Pick C_1-Like$_1$(NPC_1L_1) protein was as follow:

Primer	Primer sequence
NPC_1L_1(F)	5'-TAC TTC CAC AAC AGT GTG TC-3'
NPC_1L_1(R)	5'-ATC ACA GCA AGT ACT GAC AT-3'
BETA(ACTB)F	5'-CTGACTGACCGCGTTACTCC-3'
BETA(ACTB)R	5'-ACCATCACACCCTGATGTCTG-3'

This protein called carrier protein of cholesterol. Isolation of RNA from the small intestinal tissue and RT-PCR were done by GF-1 total RNA extraction kit and Thermo Finisher Scientific Inc.

Statistical analysis: Results were expressed as mean±SE. All data from the experiment were examined statistically by one-way anova with computerized SPSS package program (SPSS 16.00 software for Windows). A p-value ≤0.05 was considered statistically significant **(Snedecor and Cochran 1980)**.

Table (1): composition and calculated analysis of grower and finisher diets:

Ingredients %	Grower diet			Finisher diet		
	G_1(PF.)	G_2(SO.)	G_3(MIX.)	G_1(PF.)	G_2(SO.)	G_3(MIX.)
Yellow corn	58.2	58.5	58.3	64	64	64.2
Soybean meal 47%	29.4	29.2	29.3	23.2	22.8	228
Corn gluten	5.5	5.5	5.5	6	5.5	6.5
SO. Oil	0	3	1.5	0	3.2	0.95
Polyfat oil	3.5	0	1.7	4	0	2.6
Limestone	1.1	1.5	1.2	1.2	1.5	1.3
Dicalcium phosphate	1.7	1.7	1.7	1.2	1.2	1.2
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.3	0.3	0.3	0.3	0.3	0.3
DL-methionine	0.12	0.12	0.12	0.12	0.12	0.12
Dl-lysine	0.1	0.1	0.1	0.1	0.1	0.1
calculated analysis						
CP%	20.9	20.9	20.9	19.02	19.15	19.7
ME, Kcal/kg	3101	3108	3103	3195	3196	3194
Calcium %	1	1.02	0.97	0.93	0.9	0.92
Available P %	0.46	0.46	0.46	0.36	0.36	0.36

* Each 3 kg of Vit. & Min. Mixture (Premix)contains: Vit. A 12000,000 IU, Vit. D3 2000,000 IU, Vit. E 10,000 mg, Vit. K3 2000 mg, Vit. B1 1000 mg, Vit. B2 5000 mg, Vit. B6 1500 mg, Vit. B12 10 mg, Pantothenic acid 10,000 mg, Niacin 30,000 mg, Folic acid 1000 mg, Biotin 50 mg, Choline 300,000 mg, Manganese 60,000 mg, Zinc 50,000 mg, Copper 10,000 mg, Iron 30,000, Iodine 1000 mg, Selenium 100 mg, Cobalt 100 mg, Ca CO3 to 3,000 g.

Table(2): Effect of calcium salts of fatty acids on antioxidants, lipid peroxidation and body weight (M±STE).

Groups	G1	G2	G3
MDA	1.607±0.2654^{a}	3.534±0.5695^{b}	2.956±0.6102^{ab}
Blood GSH (mg/dl)	17.80±1.203^{a}	10.887±0.886^{b}	16.033±1.675^{ac}
Tissue GSH (mg/g.Tissue)	19.602±1.417^{a}	11.853±1.507^{b}	16.568±1.855^{ac}
Blood GPx (mU/mL)	3553.48±464.39^{a}	4660.40±416.58^{a}	4666.72±455.1^{a}
Tissue GPx (U/g.T)	10330.07±441.05^{a}	10722.73±608.45^{a}	10178.10±757.^{a}
Weight at day 22 (gm)	685±33.813^{a}	680±38.643^{a}	621.67±26.225^{a}
Weight at day 42 (gm)	2070.5±62.0259^{a}	2198.5±45.147^{a}	2290.5±53.234^{a}

Means with same superscript letters in each column are not significantly differed (P>0.05), Means with different superscript in each column are significantly differed (P≤0.05).

Table (3): Effect of calcium salts of fatty acids on lipid profile, gene expression, serum Ca^{+2} and P(M±STE).

Groups	G1	G2	G3
Serum total Cholesterol (mg/dl)	103.801±6.82^{a}	107.978±6.18^{a}	101.2368±5.34^{a}
Serum LDL Cholesterol (mg/dl)	52.048±5.721^{a}	49.609±6.27^{a}	51.989±6.305^{a}
Serum HDL Cholesterol (mg/dl)	28.196±1.209^{a}	30.99±1.44^{a}	27.58±2.059^{a}
Serum TAG (mg/dl)	36.883±5.35^{a}	49.679±6.75^{ab}	61.775 ±5.44^{b}
Gene expression of NPC1L1	0.5113±0.042^{a}	1 ±0^{a}	0.3135±0.1267^{a}
Serum Ca^{+2} (mg/dl)	4.84±0.27^{a}	4.75±0.28^{a}	4.35±0.28^{a}
Serum P (mg/dl).	2.45±0.13^{a}	2.58±0.19^{a}	2.71±0.16^{a}

Means with same superscript letters in each column are not significantly differed (P>0.05), Means with different superscript in each column are significantly differed (P≤0.05).

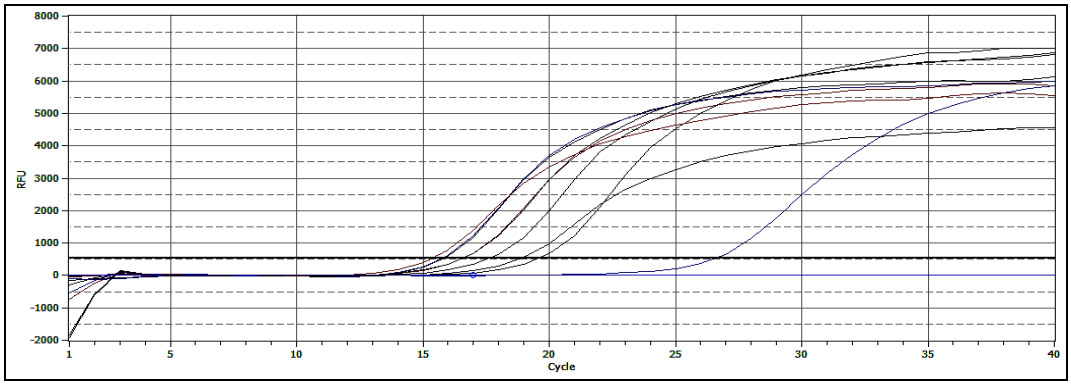

Figure (1): Amplification for beta gene.

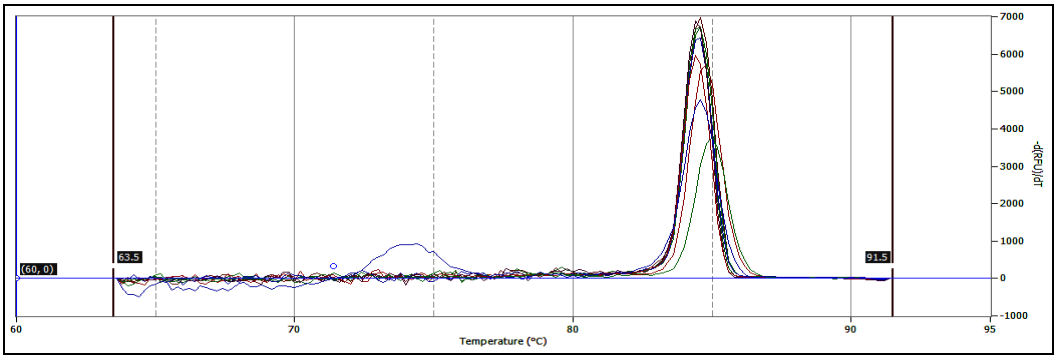

Figure (2): Melting for beta gene

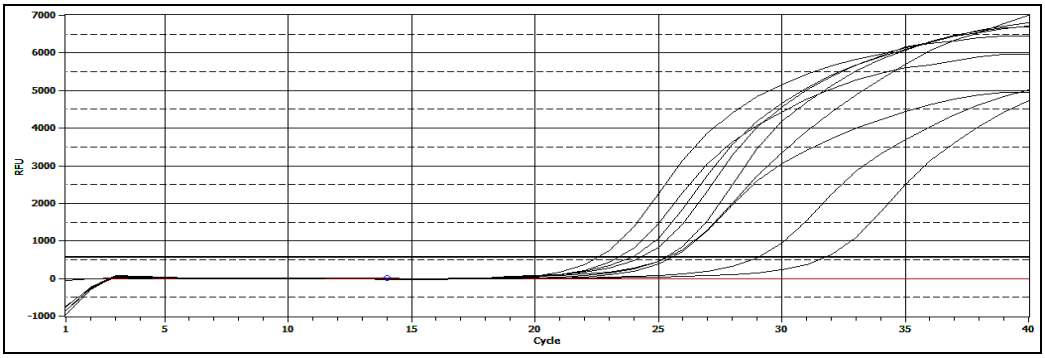

Figure (3): Amplification for NPC_1L_1.

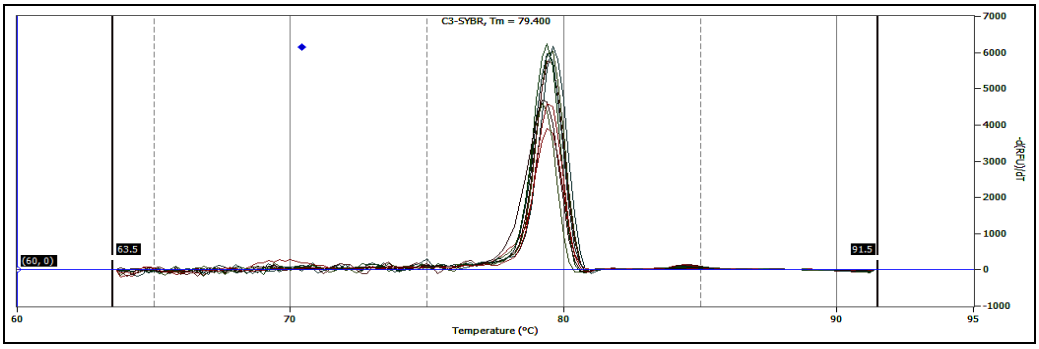

Figure (4): Melting for NPC_1L_1.

RESULTS AND DISCUSSION

The present work addresses the problem of the replacement of vegetable oils in feeding mixtures with Ca-salts of fatty acids in the fattening of broiler chickens.

1)Growth Performance

Results showed In table (2) indicate that the total live weight and feed intake were not significantly affected by calcium salts of fatty acids while there was an increase in feed conversion ratio FCR(=2) ,while the mixture of PF+SO lead to improve the total live weight and decrease FCR(=1.8) more than poly fat alone or soybean oil alone (FCR=1.9) at the end of day 42 of age, also had no effect at day 22 .

These results matched with those of **Mehmet et al., (2005**) who reported that final body weight was not significantly altered by dietary fat sources. Moreover, the obtained results agree with those of **Tabeidian & Sadeghi (2006**) who reported that Inclusion of fat powder calcium salts of fatty acid had no significant effect on feed intake. Feeding different levels of fat powder significantly ($P<0.05$) decreased weight gain and significantly ($P<0.05$) increased feed conversion ratio in 7-21 and 7-49 days old broiler chicken, **MALÁ et al., (2004)** also showed that weight was lower in chicks that had been fed with Ca-salt of fatty acids and he concluded that the energy value of Ca-salts of fatty acids is lower than that of commonly used fat and oil. While **Mutassim (2013**) reported that the inclusion of dry fat and 3 kg of yeast culture/t improved body weight gain and feed conversion .

The obtained results in this research may be due to the fact that long-chain unsaturated fatty acids have greater ability to form micelles, they may act synergistically in the absorption of saturated fatty acids when mixed with them (**Ferreira 1999**).

Free fatty acids affect absorption negatively and, consequently, also they decrease the nutritive value of the fat. The ratio between free fatty acids and the intact triacylglycerol is important, since supplements with free fatty acids are absorbed less efficiently than the free fatty acids in form of triacylglycerol. The reason for this is that monoacylglycerol are essential to incorporate insoluble fatty acids in the micellar complex. There are no enough monoacylglycerols to combine with all free fatty acids when they are submitted as the only fat source and absorption is then impaired (**Fats in animal feeds 1985 and Blanch et al., 1995**).

2)Antioxidants And Lipid Peroxidation:

Results in table (2) showed that dry fat could improve the antioxidant state and reduce lipid peroxidation as there was a significant increase in GSH content ($P<0.001$), GPx activities and reduction in serum MDA more than oil sources. Poly fat alone or mixed with SO had a positive effect on GSH content and GPx activities in both blood and liver tissue and serum MDA. These results were supported by **Pechova et al., (2006**) who noticed a higher total anti-oxidant status of cows serum when fed calcium salts of rapeseed oil while glutathione peroxidase did not affected, **Marja et al., (1984**) demonstrated that the type of dietary fat can affect the plasma GPx activity in chicks. But **MALÁ et al., (2004**) showed that Ca-salts of fatty acids have no negative effect on chickens' state of health and the quality of carcass.

On the opposite side **Serdar et al., (2005)** also found that there was no significant differences in GPx activities when chicks were fed on different fat sources but the corn oil caused significant increases in liver level malondialdehyde as compared with the other fat (butter, margarine, olive oil, sunflower oil). Moreover **Halamíčková et al., (2006**) found an opposite opinion as reported that the content of malondialdehyde in the thigh muscles was significantly lower in chickens fed soybean oil that in chickens fed Ca-salts of rapeseed and soy fatty acid oil, this was due to higher supply of fat with increased proportion of saturated fatty acids.

The effect of palm oil may be attributes to its antioxidant properties. Palm oil is rich in antioxidant vitamins tocotrienols and unsaturated analogue of tocopherols. Tocotrienols had a hypocholesterolemic effect probably through the inhibition of cholesterol synthesis **(Choi et al., 1993 and Karaji-Bani et al., 2006).**

This confliction may be due to the fact that the rate of lipid peroxidation depends on the fat level, profile of fatty acids and the specific storage conditions (**Zanini et al., 2006**).

3)Lipid Profile :

Results in tables (3) and figures (1,2,3,4) showed that there was a non significant differences in serum cholesterol, HDL and LDL while there was a significant decrease in TAG in PF group when compared with the mixture (G3) (*P*=0.05), these results was supported by RT-PCR that revealed a decrease in NPC1L1 expression (cholesterol carrier protein that is responsible for absorption of cholesterol from the small intestine) in G1 (had the lowest expression) and G3 than control group. **Tabeidian and Sadeghi (2006)** have the same opinion while reported that no significant differences were observed in plasma triacylglycerol, cholesterol, HDL and LDL when calcium salts of fatty acid included in chicken diet, **Mala et al., (2004**) also matched with these results and showed no significant differences in these parameters in plasma but **Mutassim (2013**) was in a partial disagreement with the results in the present research when detected that there was a significantly (P=0.05) higher concentration of blood serum cholesterol was found in chicks fed dry fat but the inclusion of dry fat with yeast culture positively affected the carcass characteristics by reducing the abdominal fat and blood cholesterol level.

The significant increase in serum triacylglycerol concentration in G3 may due to intensive lipid metabolism and transport and the significant decrease in G1 may be due to unbalanced ratio between free fatty acids and intact triacylglycerol.

4) Minerals

Minerals are essential for broiler growth and they are involved in many digestive, physiological and biosynthetic processes within the body. Calcium is mainly needed for the ossification of bones, regulation of muscle activity and catalization of enzyme and hormone systems while phosphorus is an important constituent of nucleic acids and phospholipids (**Underwood & Suttle 1999**).

Results in table (3) showed that there was no significant differences in serum Ca and inorganic P when dry fat was included in broilers diet. The obtained results agreed with those of **Mutassim (2013)** who found that there was no significant differences in serum Ca+2 and inorganic P when dry fat incorporated in broilers diet.

Glutathione peroxidase is a major selenium containing enzymes found in mammalian cells which catalyzes the

degradation of various peroxides by oxidizing glutathione and protect the cells from oxidative damage. Supplementation of selenium have a profound influence on GPx activities, indicating the importance of mineral selenium as it enhances the antioxidant status of the bird by increase in the activity of GPx in the serum and liver tissues of broilers. The synergistic effect of vitamin E and selenium has an in vivo antioxidant effect, which can protect against oxidative damage and lipid peroxidation of PUFA (Polyunsaturated Fatty Acids) **(Stanley et al., 1974 and Parris 1997).**

GPx was used as selenium index and there was no significant differences between groups while Poly fat® group and the mixture SO + PF group show the highest GPx activities as shown in table (2). So adding calcium salts of fatty acids (PF) had no effect on selenium absorption.

Harrison and Conrad (1984) found that apparent selenium absorption was maximum when dietary calcium was 0.8% of dry matter intake. Amounts of dietary calcium less or greater than 0.8% of dry matter intake reduced apparent selenium absorption. Dietary calcium quantitatively affected apparent selenium absorption in amounts of nutritional significance when selenium was provided from natural feed stuffs. Moreover **Holben et al., (2002)** was in the same side with the results in the present research when reported that selenium status did not differ with calcium supplementation in human.

CONCLUSION

In conclusion, oxidative stress which is the major cause of concern as result of rapid growth rate in broiler chickens can be minimized by the supplementation of dry fat that ameliorate the stress and thus can be safely used as seen in the present study. To gain heavy weight, improved antioxidant state and achieve low peroxidation level it is recommended to use a mixture of calcium salts of fatty acids + soybean oil in broilers diet instead of using ordinary oils alone.

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الملخص العربي

تأثير املاح الكالسيوم للأحماض الدهنيه على النمو والتغيرات الكيميائية في دجاج التسمين

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يتم اضافه الزيوت لأعلاف الدواجن بهدف تعويض الطاقة اللازمة وزيادة وزن الطائر ولكن يواجه المستهلك والمربي علي حد سواء بعض المشاكل نتيجة استخدام الزيوت في صورتها السائلة ، لذا يتم الآن استخدام تلك الزيوت ولكن في صوره صلبه باستخدام أملاح الكالسيوم للأحماض الدهنيه ، فكانت التجربة علي ٦٠ كتكوت من عمر ١٠ أيام ل عمر ٤٢ يوم تم تقسيمهم الي ٣ مجموعات علي حسب نوع الدهون المقدمة للطائر (٢٠ كتكوت للمجموعه) لمعرفه تأثير أملاح الكالسيوم للأحماض الدهنيه على النمو والتغيرات الكيميائية .

المجموعه الاولي : المكونات الاساسيه للعلف مضافا اليها أملاح الكالسيوم للأحماض الدهنيه (البولي فات).

المجموعه الثانيه : المكونات الاساسيه للعلف مضافا إليها زيت الصويا.

المجموعه الثالثه : المكونات الاساسيه للعلف مضافا إليها مزيج من زيت الصويا والبولي فات.

وفي نهاية التجربة تم جمع عينات من الدم وانسجه الكبد والأمعاء الدقيقة وبعد قياس معامل التحويل وبعض مضادات الاكسده في الدم وانسجه الكبد بالاضافه لقياس صوره عامه عن نسبه الدهون في الدم والانسجه باستخدام التعبير الجيني لامتصاص الكولسترول في الأمعاء الدقيقة وقياس الكالسيوم والفسفور في الدم كانت المجموعات المضاف إليها أملاح الكالسيوم للأحماض الدهنيه هي الأفضل على الإطلاق.