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IMPACT OF PUMPKIN OIL AND COPPER AND THEIR SYNERGISTIC EFFECTS ON PRODUCTIVE PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF WEANING MALE RABBITS

M.E. El-Speiy[1]; M.A. El-Sawy[1]; T.A. Sadaka[1]; M.R. Habib[1]; B.M. Abou-Shehema[1]; H.A. Shahba[1] and Asmaa[2] Sh. Elnaggar

1- *Rabbit Res. Dept., Anim. Prod. Res. Ins., Agric. Res. Center, Egypt.*
2- *Dept. of Anim. and Poultry Prod., Fac. of Agric., Damanhour Univ., Egypt.*

e. mail: *Mohamed.elspeiy@yahoo.com; Elsawy1966@gmail.com; Doctor.tarek@gmail.com; Mrh20152020@gmail.com; Bahaa.abou-shehema@arc.sci.eg; Hossam-shahba2009@arc.sci.eg; asmaaelnaggar@yahoo.com*

ABSTRACT

This study was conducted to assess the influence of pumpkin seed oil (PSO), copper sulfate ($CuSO_4$) and their synergistic effects on the productive performance, physiological response and economic efficiency of weaning rabbits. Sixty weaned Californian male rabbits at 5 weeks of age with an average initial weight of 582.83 ± 34.47 g were randomly distributed into four experimental groups (15 each). The first group was fed the basal diet and served as a control (G1). While, the 2nd (G2), fed the basal diet supplemented with 5 ml of PSO/kg diet; the 3rd (G3), fed the basal diet supplemented with 200 mg $CuSO_4$/kg diet and the 4th (G4), fed the basal diet supplemented with 5 ml PSO and 200 mg $CuSO_4$/kg diet. Results showed that rabbits in G3 and G4 had significantly higher final body weight (FBW), total body gain (TBG) and performance index (PI), while having lower feed consumption (FC) and feed conversion ratio (FCR) compared with G1. Rabbits in G3 recorded significantly higher values of organic matter (OM) digestibility compared to the other groups. Also, dry matter (DM) and crude protein (CP) digestibility were significantly improved in G3 compared with either G1 or G2 without significant when compared with G4. Hot carcass and total edible part % were significantly ($P \leq 0.05$) increased in all treated groups especially G4 compared to G1. While, total protein (TP), globulin (Glb), high-density lipoprotein (HDL), and were significantly higher ($P \leq 0.05$) in G3 and G4, while, aspartate aminotransferase (AST), creatinine (CR),triglycerides (TG), low-density lipoprotein (LDL) and very low-density lipoprotein (V-LDL) concentrations decreased compared with G1. All rabbits in supplemented groups recorded significantly ($P \leq 0.05$) higher values of plasma growth hormone (GH), thyroid stimulating hormone (TSH) and plasma thyroid hormones (T_3 and T_4) compared with

G1. Plasma IgG and IgM concentrations, total antioxidant capacity (TAC), and superoxide dismutase (SOD) significantly increased while, malondialdehyde (MDA) was decreased in all supplemented groups compared with G1. Economic efficiency was significantly improved in G3, followed by G4, while the worth value of economic efficiency found in G2 which significantly decreased compared with the control group.

Conclusion: *Pumpkin oil showed an improvement in productive performance and various blood characteristics, as well as copper, but copper had a great advantage in improving economic efficiency, especially when added alone, unlike pumpkin oil, which showed a decrease in economic efficiency compared to the control.*

Keywords: Rabbits, pumpkin seed oil, copper sulfate, growth, digestibility, blood biochemical, economic efficiency.

INTRODUCTION

The European Union has forbidden the use of most antibiotic growth promoters as feed supplements due to the development of microorganisms and residues in tissues (Ronquillo and Hernandez, 2017). On the other hand, medicinal plants and essential oils are more acceptable and safer for health because they have antimicrobial properties and improve the digestibility of nutrients (Hajati *et al*., 2015). Rabbits are described by their smaller body size, faster growth rate, more fiber nutrition, higher meat quality, early maturity, and high potential for genetic selection compared to other farm animals (Kumar *et al*., 2018). In addition, rabbits achieve an important balance between ruminants and mono-gastric animals and can gainfully use cellulose-rich feed with a quantity containing nearly 20% grain (Attia *et al*., 2012). The importance of the rabbit as a farm animal, mainly for meat-yielding, means that the progression of the gastrointestinal tract, especially the mucous membrane (villi for absorption) and the sub mucosa (glands) for enzymatic digestion is much more important (Elnasharty *et al*., 2013).

Pumpkin belongs to the family Cucurbitaceae is one of the best-known natural antioxidants (Shaban and Sahu, 2017) and an abounding natural source of protein, phytosterols, and omega-3 fatty acids (Perez Gutierrez, 2016), as well as polyunsaturated fatty acids which contain approximately 80% unsaturated fatty acids (principally oleic and linoleic acids) (Rojas *et al*., 2019), vitamins like carotenoids and tocopherol, and micro-elements such as zinc (Makni *et al*., 2008). Dietary antioxidant factors are of particular importance for the maintenance of growth, reproduction and immune proficiency in animal production by lessening the unfavorable effects of free radicals and toxic metabolites in animals (Peter, 2007).

Copper (Cu) is a vital component of aquatic physiological processes and is required by most living organisms. Deficiency of Cu in the diet has been associated with metabolic changes in numerous animals, including farm animals (Güçlü *et al*., 2008). Feeding diets containing copper in the shape of copper sulfate ($CuSO_4$) have been proven to improve performance (Helal *et al*., 2018). When administered, this element has been revealed to be important for growth progress with antibacterial action (Charmaine and Hans, 2021). Copper appears as a cofactor of many antioxidant enzymes that are influence animal immunity of (El-Hady and Mohamed, 2019).

Therefore, the objectives of this study were to examine the effects of pumpkin seed oil (*Cucurbita pepo) and* copper sulfate and evaluate their synergistic effects on growth performance, digestibility of nutrients, carcass traits, blood biochemical parameters, and economic efficiency in weaning male rabbits**.**

MATERIALS AND METHODS

***Animals and experimental design*:**

The study was carried out during season production (winter) at a private farm in Qalyubia Governorate, Egypt. Sixty Californian male rabbits, five weeks old were randomly divided into four groups (15 each); with an average body weight (BW) of 582.83 ± 34.47 g Animal care, the experimental period lasted seven weeks. The first group (G1) was fed a basal diet and served as a control group. The 2nd group (G2) was fed a basal diet supplemented with 5 ml of pumpkin seed oil (PSO)/kg diet, the 3rd group (G3) was fed a basal diet supplemented with 200 mg of copper sulfate ($CuSO_4$)/kg diet, respectively. While the 4th group (G4) was fed a basal diet supplemented with 5 ml PSO plus 200 mg of $CuSO_4$/kg diet.

The rabbits were housed individually in commercial cages (55×60×30 cm) that were equipped with automatic feeders and drinkers. The entire house was well-ventilated with both natural windows and electric fans and illuminated to 16:8 hrs light-dark cycle with natural and fluorescent light. According to NRC (1977), the basal experimental ration was formulated and pelleted to cover the nutrient requirements of growing rabbits (Table 1). Feed was allowed to a standard pelleted diet at all times containing 17% CP, 2.56% EE, 13.04% CF and contains 2500 Kcal/kg-ration DE.

Table 1. Ingredients and chemical composition of the experimental diets

Ingredients	**Control diet (Kg)**
Barley	125
yellow Corn	80
Wheat bran	180
Alfa alfa	180
Soybean meal 44%	180
Hay	194
Premix*	3
Sodium chloride	3
Di-Calcium-phosphate	17
Limestone	4.9
Lysine	0.95
DL-Methionine	2.15
Molasses	30
Total	**1000**
Crude protein	17.00
DE kcal/kg	2500
Ether extract %	2.56
Crude fiber %	13.04
Ca %	1.10
P %	0.51

* 3 kg premix contains of vitamins and minerals. Vit. A= 2.000.000 IU, Vit. B_1= 0.33 g, Vit. B_2= 1.0 g, Vit. D_3= 150.000 IU, Vit E= 8.33 g, Vit. K_3= 0.33 g, Pantothenic acid=3.33 g; Nicotinic acid=30.00 g; Vit. B_6=2.00 g; Vit. B_{12}=1.7 mg, Folic acid= 0.83 g, Biotin=33 mg, Cu=0.5 g, choline chloride=200 mg, Mn=5.0 g, Fe=12.5 g, Mg=66.7 mg, Co=1.33 mg, Se=16.6 mg, Zn=11.7 g, Iodine=16.6 mg and Anti-oxidant=10.0 g).

Tested materials:

1- ***Pumpkin seed oils***

The active substances found in PSO are presented in Table 2. Pumpkin seed oil (PSO) was analyzed at the Regional Center for Food and Feed. This center obtained international accreditation according to the international standard ISO/IEC 17025 from A2LA. The fatty acid composition was determined according to Cocks and Van Rede (1966). Phenolic acids were assayed according to Mateos *et al*., (2001). Tocopherols were quantified according to Kamal-Eldin

Table 2. Chemical composition of pumpkin seed oil

Major active substances	The value
Saturated fatty acids %	22.5±0.3
Monounsaturated fatty acids%	45.1±0.5
Polyunsaturated fatty acids%	32.4±0.7
Oleic acid%	40.1± 2.8
Linoleic acid%	37.97±3.6
Palmitic acid%	18.77±0.8
Tocopherols, mg/100 g	80.65±7.5
B-sisosterol, mg/100 g	874.61±23.54
Phenolic acids mg/100 g	5.87±0.02

et al., (2000). Analysis was executed according to Nyam *et al*., (2009). The dose used in our study according to Bakeer (2021).

2- Copper sulfate:

Copper sulfate is an inorganic compound and used as a dietary, formula: $CuSO_4$ $5H_2O$, molar mass: 159.47 g/mol, soluble in water ($CuSO_4$ $5H_2O$, 98% ($CuSO_4$; contains 25% Cu), was purchased from El-Gomhouria Company for Chemical, Drugs and Medical Instruments, Alex, Egypt. Rabbits' basal diet containing approximately 12 mg Cu/kg diet (7 mg from premix and 5 mg from other ingredients). While, the dose used in our study (200 mg $CuSO_4$/kg diets) was supplemented above the proportion of copper in the basal diet according to (Easa *et al*., 2018).

Growth performance traits:

The average total body gain (TBG) was calculated as follows:

$$TBG\ (g) = (\text{Final live BW} - \text{Initial live BW}).$$

The difference between the weight of the feed offered and the weight of the remaining on the same day of weighing the animals were used to calculate feed consumption (FC). The ratio of animal FC to TBG was calculated as the feed conversion ratio (FCR). Performance index (PI) was calculated according to (Easa *et al*., 2018).

$$PI\ (\%) = [\text{Final live BW (kg)}/\ FCR] \times 100$$

Digestibility trial:

A digestibility trial was done after a 10-day adaptation period, 6 male rabbits per treatment individually housed in metabolic cages that allow feces collection. Quantitative collection of feces started 24 h after offering the daily feed. Feces of each rabbit were collected once a day at 9.00 am and FC was recorded every day in the morning for four days as the collection period. The collected samples of feces and feeds were pooled and stored at -18º C until analysis. Fecal samples were dried at 60º C for 72 h and ground through a 1 mm screen on a Wiley grinder. Digestibility coefficients were determined and expressed on dry matter (DM) basis using the ((nutrient intake - nutrient voided = retained) / intake) × 100. Feed and fecal samples from experimental groups were chemically analyzed according to AOAC (2000). NFE was calculated from the difference between DM and other components.

***Carcass characteristics*:**

At the end of the experimental growing period**,** six rabbits were selected for carcass ratings based on an average of each treatment. The rabbits were fasted with free water for 12 hours before slaughtering. Rabbits were weighed prior to slaughter**,** slaughtered for complete emptying, skinned, and gutted. The trimmed carcass free of internal organs (carcass weight) was weighed. The gutted carcass, including the liver, heart, and kidneys, was weighed. Carcass yields were calculated as a percentage of the live body weight (LBW) of the rabbits before slaughter. Furthermore, the percentages of the dressing and giblets were calculated as described by Zweil *et al*., (2019) as follows:

(1) Giblets weight (g) = kidneys weight (g) + heart weight(g) + liver weight (g);

(2) Dressing (g) = carcass weight (g) + giblets weight (g).

Blood sampling and analyses:

At the end of the experiment, blood samples were drawn from the ear vein and centrifuged at 3000 rpm for 15 minutes at 4 °C. Plasma was stored at –20 °C until analysis. Total protein (TP), albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), activities, urea (BU), creatinine (CR), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL)were determined using appropriate kits (Bio-diagnostics, Recycling Crusher-SBM®, www. Bio-diagnostic.com). Meanwhile, globulin (Glb) was calculated by the difference between total protein (TP), and albumin (Alb). While, low-density lipoprotein (LDL**)** was calculated using the formula:

LDL (mg/dl) =TC –{HDL+ (TG/5)}), which explained by William *et al.*, (1972). Also, very low-density lipoprotein (VLDL) was calculated as one-fifth of triglycerides (Friedewald *et al.*, 1972). For growth hormone (GH) by radioimmunoassay technique was performed according to Chu (2003). Thyroid stimulating hormone (TSH) as well as thyroid hormones, triiodothyronine (T_3), and tetraiodothyronine (T_4) were determined according to Carayon *et al*., (2002). Lipase activity and amylase activity were taken on a Chem-Well 2900 (T) automated analyzer (United States) with the use of the appropriate HUMAN reagent kits (Germany). Plasma immunoglobulin's (IgG and IgM) were determined using ELISA technique. IgG value was determined by IgG ELISA kit (Catalog No: MBS043814). IgM value was determined by IgM ELISA kit (Catalog No: MBS700823). Blood plasma totals antioxidant capacity (TAC), superoxide dismutase (SOD), and malondialdehyde (MDA) were determined using appropriate kits (Bio-diagnostics, Recycling Crusher-SBM®, www. Bio-diagnostic.com).

Economic efficiency:

The economic efficiency and relative economic efficiency were estimated according to El-Speiy *et al*., (2015) dependent on the (FBW) and the price of (kg rabbit, kg diet, weaned rabbits, and additives).

Statistical analysis:

Data from all response variables were subjected to one-way analysis using (SAS, 2002). Variables having significant differences were compared using Duncan's Multiple Range Test (Duncan, 1955). The statistical model used was as follows:

$$Y_{ij}= \mu+T_i+e_{ij},$$

Where, Y_{ij}= The dependent variable, μ= the overall mean; T_i= The effect of treatments and e_{ij}= The random error.

RESULTS AND DISCUSSION

Growth performance:

The impact of PSO and $CuSO_4$ and their synergistic effects on the BW, TBG, FC, FCR and PI are presented in Table 3. Rabbits in groups G3 and G4 recorded significantly ($P\leq0.05$) the highest FBW and TBG while significantly ($P\leq0.05$) decreasing FCR compared with either G1 or G2. In addition, G3 and G4 recorded significantly ($P\leq0.05$) lower FC compared with the rabbits in G1 without significant when compared with G2. The performance index was significantly ($P\leq0.05$) improved in all treated groups and showed a synergistic effect in G4. It greatly means that despite the groups of the G3 and G4 diets consuming lower feed and at the same time, heaviest final body weight and TBG than the other groups, this favorable effect would also be reflected in the FCR. Regarding PSO,

Table 3. Impact of pumpkin seed oils (PSO), copper sulfate ($CuSO_4$), and their synergistic effects on the productive performance of weaning male rabbits during 5–12 weeks of age

Tr	Parameters					
	IBW, g	**FBW, g**	**TBG, g**	**FC, g**	**FCR**	**PI (%)**
G1	583.1	1953.1^{c}	1370.0^{c}	5551.0^{a}	4.05^{a}	48.22^{d}
G2	583.7	2059.8^{b}	1476.1^{b}	5452.8^{ab}	3.69^{b}	55.82^{c}
G3	582.6	2150.0^{ab}	1567.4^{ab}	5354.8^{b}	3.42^{c}	62.87^{b}
G4	581.9	2223.3^{a}	1641.4^{a}	5354.6^{b}	3.26^{c}	68.20^{a}
MSE	34.47	84.94	79.82	103.41	0.041	5.26
Sig.	NS	*	*	*	*	*

$^{a-b-c-d}$: Values in the same column with different superscripts differ significantly ($P \leq 0.05$), NS= not significant, Tr = Treatments; G1= Control; G2= Rabbits were treated with 5 ml of PSO/kg diet; G3= Rabbits were treated with 200 mg of $CuSO_4$/kg diet; G4= Rabbits were treated with 5 ml of PSO + 200 mg of $CuSO_4$/kg diet; IBW= Initial body weight; FBW= Final body weight; TBG= The average total body gain; FC= Feed consumption; FCR= Feed conversion ratio; PI = Performance index.

results disagree with those obtained by Ragab *et al*., (2013a) who reported that growing rabbits supplemented with 5 g/kg diet of PSO showed no significant effect on FBW, TBG, and FCR, while FI significantly increased compared to the control. Meanwhile, the results of our study agree with those of Bakeer (2021), who reported that supplementation diets with PSO increased rabbit FBW at a dose of 5 g PSO/kg diet. This improvement has been clarified by Rojas *et al.,* (2019) who suggested that these improvements are probably attributed to unsaturated fatty acids, carotenoids, and tocopherols in PSO, which are essential for animal metabolism.

The current results are in agreement with many studies that have been conducted to evaluate adding copper to rabbit diets, Adu and Egbunike (2009) found that Cu could be used as a growth promoter in rabbit diets without negative effects. Another research study on other forms of Cu such as nano-copper by Refaie *et al*., (2015) revealed that supplementing weaning rabbit diets with nano-copper at dose 50 or 70 mg /kg diet led to an increase in FBW and PI compared to control. Also, Easa *et al*., (2018) recorded that NZW rabbits utilized a diet with $CuSO_4$ or Cu-nano showed significant greater growth performance compared to control rabbits. Finally, Charmaine and Hans (2021) documented that Cu can be included in growth promoting for weaning and growing pigs by reducing post weaning diarrhea and enhancing growth performance because Cu plays an important role in the digestive tract and bacterial count and changes the 3-dimensional structure of bacterial proteins, which prohibits bacteria from exerting their pathogenic action and might disrupt enzyme structures and functions of bacteria by binding to sulfur or carboxylate-containing groups and amino acid groups of proteins.

Nutrient digestibility:

The impact of PSO and $CuSO_4$ and their synergistic effects on the nutrient digestibility of the diets of weaning male rabbits is illustrated in Table 4. Rabbits fed a diet supplemented with 200 mg of $CuSO_4$/kg recorded significantly higher values of OM compared with other experimental groups. While, DM and CP digestibility were significantly improved in G3 and G4 compared to G1 or G2.

On the other hand, the other nutrients' digestibility (CF % and NFE %) were not significantly affected by the addition of any treatment. It appears from the results of digestibility coefficients that there is no synergistic effect in G4 while, treated with $CuSO_4$ alone in G3 showed a similar or superior effect when compared to G4. Concerning PSO, Ragab *et al.*, (2013a**)** found that digestibility coefficients in rabbit fed diets supplemented with PSO significantly improved the digestibility of DM, OM, CP, CF, EE and NFE.

Table 4. Impact of pumpkin seed oils (PSO), copper sulfate ($CuSO_4$) and their synergistic effects on nutrient digestibility (%) of diets of weaning male rabbits during 5–12 weeks of age

Tr	Parameters (%)					
	OM	DM	CP	EE	CF	NFE
G1	67.24^{b}	63.25^{b}	73.25^{b}	75.19^{a}	24.65	72.16
G2	68.86^{b}	64.82^{b}	73.98^{b}	76.43^{a}	25.15	73.02
G3	70.16^{a}	67.52^{a}	76.19^{a}	74.81^{ab}	24.29	72.19
G4	68.99^{b}	65.66^{ab}	74.55^{ab}	73.99^{b}	2482	72.69
MSE	6.14	6.03	6.85	7.04	2.31	5.81
Sig.	*	*	*	*	NS	NS

a-b: Values in the same column with different superscripts differ significantly ($P \leq 0.05$). Tr = Treatments; G1= Control; G2= Rabbits were treated with 5 ml of PSO/kg diet; G3= Rabbits were treated with 200 mg of $CuSO_4$/kg diet; G4= Rabbits were treated with 5 ml of PSO + 200 mg of $CuSO_4$/kg diet; OM= Organic matter; DM= Dry matter; CP= Crude protein; EE= Ether extract; CF= Crude fiber; NFE= Nitrogen free extract.

Supplementation with PSO improves caecal microbial balance by increasing the utilization of free volatile acids and decreasing pH values. Pumpkin seed oil improves the digestibility of nutrients and may be related to improved microflora population, mucosal immunity, and growth performance (Falcao-e-Cunha *et al*., 2007). Also, volatile fatty acids play a direct role in bacterial cell integrity (Maertens *et al*., 2006). The present results are in correspondence with those obtained by Easa *et al*., (2018) who documented that supplemented 200 mg copper sulfate/kg in rabbits' diets significantly improved nutrient digestibility of DM%, OM%, and CP%. Also, Abd El-Moneim *et al*., (2013) found that the digestibility of CP was insignificantly improved by supplemented 40 or 80 mg Cu per liter in drinking water. Likewise, Abd El-Azeem and Abd El-Reheem (2006) noticed that the digestibility of DM and OM were insignificantly increased for the group supplemented with copper sulfate in the rabbits' diet compared to the control group. Conversely, Attia (2003) revealed that the feeding values of TDN and DE were not improved by copper addition.

Carcass characteristics:

The impact of supplemented diets with PSO and $CuSO_4$ and their synergistic effects on carcass traits of weaning male rabbits are presented in Table 5. Hot carcass and dressing were significantly improved ($P\leq0.05$) except for kidneys in treatment groups compared with the control. On the other hand, G4 showed a synergistic effect in dressing which significantly increased compared with other treatments or the control.

Respecting PSO, our study showed significant improvements in the edible part, which contradicted the results obtained by Yakubu and Wafar (2014) who found no statistically significant differences between the edible part weights of buck rabbits supplemented with pumpkin leaf meal. A similar result was obtained by Wafar *et al*., (2017) who reported that all parameters weighed (liver, heart, spleen, and kidney) were not significantly affected by the inclusion levels of pumpkin leaf meal.

On the other hand, Ekpo *et al*., (2016) recorded that the organs (heart, lungs, and kidney) weight is not significant in pumpkin stem waste diets. Also, Ragab *et al*., (2013a) recorded that the percentages of liver, kidneys, heart, lungs, spleen, and head were nearly similar in all groups of treatment and control. Respecting $CuSO_4$, the current results confirm those conducted by Yassein *et al*., (2011) who observed that adding copper sulfate to drinking water increased dressing and carcass percentages. Likewise, Abd El-Azeem and Abd El-Reheem (2006) noticed that 150 or 300 ppm significantly improved dressing percentage and hot carcass weight (%) but, giblets weight (%) was not affected by copper addition.

The improvement in carcass measurements can be attributed to the improvement in body weight gain as well as digestibility coefficients (Zweil *et al*., 2019).

Table 5. Impact of pumpkin seed oils (PSO) and copper sulfate ($CuSO_4$) and their synergistic effects on carcass characteristics of weaning male rabbits at 12 weeks of age

Tr	Body weight, g	Carcass characteristics (%)				
		Hot carcass	Liver	Heart	Kidney	Total edible part
G1	1870.00^{c}	50.77^{b}	3.00^{b}	0.35^{b}	0.68^{b}	54.80^{c}
G2	2080.00^{b}	54.38^{a}	3.61^{a}	0.40^{a}	0.71^{b}	59.11^{b}
G3	2181.67^{ab}	55.50^{a}	3.48^{a}	0.40^{a}	0.69^{b}	60.07^{b}
G4	2260.00^{a}	57.15^{a}	3.62^{a}	0.41^{a}	0.90^{a}	62.08^{a}
SEM	124.15	4.12	0.23	0.11	0.16	3.18
Sig.	*	*	*	*	*	*

$^{a-b-c}$: Values in the same column with different superscripts differ significantly ($P \leq 0.05$). Tr = Treatments; G1= Control; G2= Rabbits were treated with 5 ml of PSO/kg diet; G3= Rabbits were treated with 200 mg of $CuSO_4$/kg diet; G4= Rabbits were treated with 5 ml of PSO + 200 mg of $CuSO_4$/kg diet.

Plasma biochemical parameters:

Liver and kidney functions:

The impact of a supplemented diet with PSO and $CuSO_4$ and their synergistic effects on some blood plasma parameters of weaning male rabbits is shown in Table 6. Significantly higher ($P \leq 0.05$) TP and Alb in groups supplemented PSO alone or plus Cu So_4compared with group supplemented with $CuSO_4$ alone or the control group. While, Glb concentration was significantly increased in group supplemented with $CuSO_4$ alone compared with the other experimental groups. Meanwhile AST and CR were significantly ($P \leq 0.05$) decreased in supplemented groups compared with the control.

On the other hand, insignificant differences were obtained among groups for ALT. Also, a synergistic effect was shown in G4 which significantly decreased BU compared with the rest experimental groups. Regarding PSO the results obtained by Ragab *et al.,* (2013a) revealed that treatment with PSO had no effect on TP, Alb, and Glb but significantly decreased ALT in rabbits. Likewise, Rouag *et al*., (2020) recorded that PSO decreased AST and ALT. On the other hand, Bakeer (2021) revealed that treating rabbits with PSO significantly improved serum TP concentration. Regarding $CuSO_4$, Helal *et al*., (2018) showed an increase in TP and BU, while insignificant ALT, AST, and CR in rabbit's plasma fed diet supplemented with Cu. Meanwhile, Adu and Egbunike (2009) showed that supplemented male growing rabbit with Cu with a range of 100-300 ppm

Table 6. Impact of pumpkin seed oils (PSO), copper sulfate ($CuSO_4$) and their synergistic effects on plasma proteins, kidney and liver function of weaning male rabbits during 5–12 weeks of age

Tr	Parameters						
	TP, (g/dl)	Alb, (g/dl)	Glb, (g/dl)	AST, (U/l)	ALT, (U/l)	BU (mg/dl)	CR (mg/dl)
G1	6.72^{c}	4.43^{b}	2.29^{bc}	52.20^{a}	48.12	30.82^{a}	1.02^{a}
G2	7.15^{b}	5.06^{a}	2.09^{c}	42.70^{b}	49.16	31.46^{a}	0.96^{b}
G3	7.29^{ab}	4.62^{b}	2.67^{a}	43.50^{b}	49.41	32.07^{a}	0.99^{b}
G4	7.40^{a}	5.02^{a}	2.38^{b}	40.60^{b}	45.03	26.48^{b}	0.91^{c}
MSE	1.14	1.07	0.09	1.04	1.07	1.52	0.02
Sig.	*	*	*	*	NS	*	*

$^{a-b-c}$: Values in the same column with different superscripts differ significantly ($P \leq 0.05$). Tr = Treatments; G1= Control; G2=Rabbits were treated with 5 ml of PSO/kg diet; G3=Rabbits were treated with 200 mg of $CuSO_4$/kg diet; G4=Rabbits were treated with 5ml of PSO + 200 mg of $CuSO_4$/kg diet; TP=Total protein; Alb=Albumin; Glb= Globulin; AST= Aspartate aminotransferase; ALT=Alanine aminotransferase; BU=Urea; CR=Creatinine.

recorded insignificant for ALT, AST, Alb, Glb, BU, and TP, but another result, obtained by Alrawi *et al*., (2017) who recorded that the copper sulfate 2.5 mg/kg BW caused a decrease in serum TP, Alb, and Glb concentrations. We can explain the results of the previous studies by saying that an increase in copper beyond the permissible limits will have bad effects on blood measurements, especially liver and kidney functions (Gembillo *et al.*, 2022).

Lipid profile:

Data in Table 7 revealed that the impact of a supplemented diet with PSO and $CuSO_4$ and their synergistic effects on the lipid profile. Triglycerides were significantly decreased in supplemented groups compared with the control and showed the highly significant decreased in G2 followed by G4 and then G3. Group supplemented with PSO alone significantly decreased TC compared with the other experimental groups, while other treated groups did not have a significant effect compared with the control. Rabbits in G4 had a synergistic effect on plasma HDL concentration, which significantly increased compared with the other groups. The values of LDL and VLDL were significantly decreased in the treated groups compared with the control, and the most significant decrease was found in the group supplemented with PSO either alone or plus $CuSO_4$. Concerning, PSO, our results harmony with those obtained by Ragab *et al.*, (2013a) who documented that a diet supplemented with 5 g of PSO/kg significantly decreased TG, TC, and LDL. Likewise, Abuelgassim and Al-Showayman (2012) reported that unsaturated fatty acids prevent the activity of intestinal acyl-coenzyme A: cholesterol acyltransferase, the rate-limiting step in the absorption of TC. The decreased serum concentrations of TC in hyperlipidemic rats might be explained in this way, as PSO is a rich source of polyunsaturated fatty acids (Ramadan *et al*., 2011). Also, Ragab *et al*., (2013b) reported lower TG, TC, and LDL levels in doe rabbits treated with PSO. Meanwhile, Bakeer (2021) noticed an increase in total lipids when the diet was supplemented with PSO. While supporting our results regarding HDL value, Ekpo *et al.*, (2019) reported that serum HDL significantly increased with an increase in pumpkin stem waste in rabbit diets. On the other hand, pumpkin stem waste significantly decreased TC, LDL, TG, and VLDL compared with control (Ekpo *et al*., 2019). Regarding $CuSO_4$, Lei *et al.*, (2017) found that Cu significantly increased plasma TG and V-LDL due to increased lipolysis. Conversely, Adu and Egbunike (2009) proved that supplementing the diet with 100-300 ppm Cu resulted in a significant decrease in cholesterol concentration. Ibrahim *et al.*, (2022) explained that Cu can regulates cholesterol biosynthesis by reducing the activity of 3-hydroxyl-3- methylglutaryl Co- (HMG-CoA) reductase.

Table 7. Impact of pumpkin seed oils (PSO), copper sulfate ($CuSO_4$) and their synergistic effects on lipid profile of weaning male rabbits during 5–12 weeks of age

Tr	Parameters				
	TG, (mg/dl)	TC, (mg/dl)	HDL, (mg/dl)	LDL, (mg/dl)	V-LDL, (mg/dl)
G1	145.8^{a}	87.40^{ab}	27.29^{d}	30.95^{a}	29.16^{a}
G2	139.0^{d}	84.50^{c}	31.79^{b}	24.91^{c}	27.80^{d}
G3	143.2^{b}	87.60^{a}	29.59^{c}	29.37^{b}	28.64^{b}
G4	140.8^{c}	86.34^{a}	33.57^{a}	24.61^{c}	28.16^{c}
MSE	1.15	1.13	1.12	1.37	0.23
Sig.	*	*	*	*	*

$^{a-b}$: Values in the same column with different superscripts differ significantly ($P \leq 0.05$). Tr = Treatments; G1= Control; G2=Rabbits were treated with 5 ml of PSO/kg diet; G3=Rabbits were treated with 200 mg of $CuSO_4$/kg diet; G4=Rabbits were treated with 5 ml of PSO + 200 mg of $CuSO_4$/kg diet; TG= Tri-glycerides; TC= Total cholesterol; HDL= high-density lipoprotein; LDL= Low-density lipoprotein; V- LDL= Very low-density lipoprotein.

Plasma hormones and digestive enzymes:

The impact of PSO and $CuSO_4$ on plasma thyroid hormones and digestive enzymes of weaning male rabbits are presented in Table 8. Results show that rabbits

Table 8. Impact of pumpkin seed oils (PSO), copper sulfate ($CuSO_4$) and their synergistic effects on plasma hormones and digestive enzymes of weaning male rabbits during 5–12 weeks of age

Tr	Parameters					
	GH (ng/ml)	TSH (nmol/l)	T_3 (nmol/l)	T_4 (nmol/l)	Lipase (U/l)	Amylase (U/l)
G1	0.031^c	0.02^c	0.74^c	2.12^b	396.40	304.20^b
G2	0.144^a	0.08^b	0.93^b	2.72^a	437.70	426.40^a
G3	0.090^b	0.08^b	0.87^b	2.66^a	434.40	405.70^a
G4	0.146^a	0.09^a	1.21^a	2.86^a	431.20	392.60^a
MSE	0.037	0.002	0.09	0.14	4.31	10.24
Sig.	*	*	*	*	NS	*

$^{a-b-c}$: Values in the same column with different superscripts differ significantly (P≤0.05), NS= not significant. Tr = Treatments; G1= Control; G2= Rabbits were treated with 5 ml of PSO/kg diet; G3= Rabbits were treated with 200 mg of $CuSO_4$/kg diet; G4= Rabbits were treated with 5 ml of PSO + 200 mg of $CuSO_4$/kg diet; GH= Growth hormone; TSH= Thyroid-stimulating hormone; T_3= Triiodothyronine; T_4= Thyroxin.

in the G4 group documented significantly (P≤0.05) higher values of TSH, T_3, T_4, and GH. Moreover, G2 and G3 recorded the same trend compared to G1. On the other hand, the G1 recorded low values of all parameters. Interestingly, G2, G3 and G4 recorded highly significant (P≤0.05) values for T_4, lipase, amylase, and GH compared to G1.

Regarding PSO Ragab *et al*., (2013b) reported that the concentrations of thyroxin (T_4) and triiodothyronine (T_3) hormones were higher in groups supplemented by PSO in rabbits which might be related to vitamin E and a phenolic compound found in PSO which has antioxidant properties by removing free radicals and inhibiting fat peroxidation and thus preserving the cell membrane of thyroid gland. Contradicted results were reported by Abdelnour *et al*., (2023) who reported that rabbits under high ambient temperatures decreased T_3 as a result of PSO increase in the diets. The present results agree with those accomplished by Al-Dabbas *et al*., (2010) who recommend that PSO raises the release of digestive enzymes and improves digestion products through the improvement of the liver enzymes poly-carboxylic acids, phosphate salts, fiber, and proteins digestive. As well as, Vertiprakhov *et al.,* (2018) reported a positive correlation between tryptic activities in the pancreas or pancreatic juice and in blood serum in chickens. Concerning blood digestive enzymes which were reported by Bakeer (2021) who showed a significant increase in serum amylase and lipase activities in rabbits fed diet supplemented with 5 g PSO/kg diet to the control. Supporting our results regarding GH, Yang *et al*., (2011) found that Cu stimulates the secretion of GH and is very important in regulating the synthesis of peptides.

The results of present study are in agreement with those of Charmaine and Hans (2021) who recorded that Cu supplementation in diet-weaning pigs' increases lipase enzyme activity in the small intestine and utilization of lipids. On the contrary, results obtained by Han *et al*., (2012) who reported in Dawley rats 'administration of 80 mg/kg BW $CuSO_4$ in the diet had no significant effects on the activities of amylase and lipase in the small intestinal compared with the control.

Immunological and antioxidant status:

The impact of PSO and $CuSO_4$ and their synergistic effects on immune function and antioxidant status are presented in Table 9. Immunoglobulin (IgG and IgM) concentrations in the blood plasma of weaning rabbits at the end of the experiment revealed that G2, G3, and G4 concentrations significantly increased compared with G1. The antioxidant parameter showed the same trend in TAC and SOD concentrations, as well as a decrease in MDA. Regarding PSO, our results are in harmony with those obtained by Negm (2018) supplementation with pumpkin significantly increased IgG and IgM levels. This was explained by the fact that it contains many antioxidants that protect the body and increase its immunity (Abdelnour *et al*., 2023).

Furthermore, Bakeer (2021) who mentioned significant improvements in the anti-oxidative parameters (SOD and TAC) of rabbits supplementing their diet with PSO. Furthermore, MDA decreased compared with control. Rouag *et al*., (2020) recorded that supplementing the diet with PSO at 4 mg/kg body weight increased numerically SOD and decreased MDA. This improvement was explained by Perez Gutierrez (2016), who noted that polyphones and flavonoids found in PSO have been capable of scavenging hydroxyl radicals and superoxide anions.

Respecting $CuSO_4$ our data confirmed that those obtained by El-Hady and Mohamed (2019) found that $CuSO_4$ significantly improved IgG, IgM, TAC, and MDA in broiler diets and related these results to the antioxidant properties of Cu which plays as a cofactor of many enzymes that are influential on the immunity of animals. Similar results were obtained by Charmaine and Hans (2021) who indicated that antioxidant capacity and humeral immune response were improved. Finally, Kushwaha *et al*., (2023) found that a Cu-supplemented to the diet had greater levels of antioxidants such as TAC and SOD. Also, IgG and total immunoglobulin levels were higher.

Table 9. Impact of pumpkin seed oils (PSO), copper sulfate ($CuSO_4$) and their synergistic effects on immune function and antioxidant status of weaning male rabbits during 5–12 weeks of age

Tr	Parameters				
	IgG, (mg/dl)	**IgM, (mg/dl)**	**TAC, (μm/l)**	**SOD, (U/ml)**	**MDA, (nmol/ml)**
G1	286.60^{c}	20.00^{c}	2.49^{c}	110.00^{b}	17.00^{a}
G2	490.90^{b}	31.20^{b}	3.25^{b}	224.75^{a}	13.33^{b}
G3	486.10^{b}	29.70^{b}	3.66^{a}	229.43^{a}	12.99^{b}
G4	502.80^{a}	35.40^{a}	3.57^{a}	239.21^{a}	11.23^{b}
MSE	18.98	3.35	0.30	7.67	3.07
Sig.	*	*	*	*	*

$^{a-b}$: Values in the same column with different superscripts differ significantly ($P \leq 0.05$). Tr = Treatments; G1= Control; G2=Rabbits were treated with 5 ml of PSO/kg diet; G3=Rabbits were treated with 200 mg of $CuSO_4$/kg diet; G4=Rabbits were treated with 5 ml of PSO + 200 mg of $CuSO_4$/kg diet; IgG= Immunoglobulin G; IgM= Immunoglobulin M; TAC= Total antioxidant capacity; SOD= Superoxide dismutase; MDA= Malondialdehyde.

Economic evaluation:

Results presented in Table 10 show that all groups supplemented with PSO, Cu, and their synergistic effects. The results showed that EE was significantly increased in group supplemented with $CuSO_4$ compared with the other experimental groups. While, G2 showed the worth EE compared with either treated groups or the control.

Results are similarly to Ragab *et al*., (2013a) who found that diet containing PSO decreased the economic efficiency of growing rabbits. Also, Easa *et al*., (2018) reported that supplementing the NZW rabbit diet with 200 mg $CuSO_4$/kg improved the economic efficiency.

The same result was obtained by Abd El-Azeem and Abd El-Reheem (2006) who noticed that the economic efficiency and relative economic efficiency were significantly improved by supplementing copper at different levels of 150 or 300 mg/kg to rabbit diets.

Conclusively, pumpkin oil showed an improvement in productive performance and various blood characteristics, as well as copper, especially measures of lipid profile, liver and kidney function, immunity, and antioxidants. However, copper had a great advantage in improving economic efficiency, especially when added alone, unlike pumpkin oil, which showed a decrease in economic efficiency compared to the control.

Table 10. Impact of pumpkin seed oils (PSO), copper sulfate ($CuSO_4$) and their synergistic effects on economic evaluation of weaning male rabbits during 5–12 weeks of age

Items	Experimental groups				MSE	Sig.
	G1	G2	G3	G4		
Final body weight, g (A)	1953.1[c]	2059.8[b]	2150.0[a]	2223.3[a]	0.085	*
body weight price, L.E. /kg (B)	90.00	90.00	90.00	90.00	-	-
Net profit, L.E./rabbit (C)**	175.78[c]	185.38[b]	193.50[a]	200.10[a]	7.64	*
Total feed consumed/rabbit, g (D)	5551.0[a]	5452.8[ab]	5354.8[b]	5354.6[b]	103.72	*
Price of kg feed+Additives cost L.E. (E)	14.50	17.00	14.51	17.01	-	-
Feed consumed cost/rabbit, L.E. (F)**	80.49[b]	92.70[a]	77.70[c]	91.08[a]	1.68	*
Weaned rabbits cost, L.E. (J)	40.00	40.00	40.00	40.00	-	-
Total cost/rabbit, L.E. (H)**	120.49[b]	132.70[a]	117.70[b]	131.08[a]	1.68	*
Net revenue L.E. (I)**	55.29[b]	52.68[b]	75.80[a]	69.02[a]	7.64	*
Economic efficiency (G)**	45.89[c]	39.70[d]	64.40[a]	52.65[b]	6.25	*
Relative economic efficiency REE(K)**	100.00	86.51	140.34	114.73	–	–

[a,b-c-d] Means in the same row with the same letters are not significantly different. MSE: Mean standard error, NS: Non-significant, *: ($P \leq 0.05$), ND: not done

*Calculations included period from 35 to 84 day-old, fixed cost = price of weaning live rabbit + electricity + vaccination …. ect, according to price in March 2022.

** C= A×B, F= D×E, H= F+J, I= C-H, G= I/H×100, K= G of treatment/G of control×100.

Price of 30 ml PSO = 15.0 LE, 1 kg of $CuSO_4$ = 50.0 LE.

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تأثير إضافة زيت اليقطين والنحاس والتأثير التأزرى بينهما علي الأداء الإنتاجي والفسيولوجي لذكور الارانب المفطومة

محمد السيد السبيعى[1]– محمد عبد العزيز الصاوى[1]– طارق أمين صدقه[1] ـ محمود رشاد حبيب[1]
بهاء محمد السيد أبو شحيمه[1]ـ حسام عبد المنعم شهبة[1] ـ أسماء شوقى محمد النجار[2]
1– معهد بحوث الأنتاج الحيوانى ـ مركز البحوث الزراعية ـ جيزة ـ مصر
2– قسم الإنتاج الحيوانى والداجنى – كلية الزراعة – جامعة دمنهور- دمنهور- مصر

أجريت هذه الدراسة لتقييم تأثير زيت بذور اليقطين وعنصر النحاس والتأثير التأزرى بينهما على الأداء الإنتاجي والفسيولوجي للأرانب المفطومة. تم توزيع 60 أرنب ذكر كاليفورنيا عمر 35 يوما بمتوسط وزن إبتدائى 582.83±34.47جم بشكل عشوائي لأربع مجموعات تجريبية بعدد 15 بكل معاملة، المجموعة الأولى غذيت علي العليقة الأساسية بدون إضافات كمجموعة مقارنة، المجموعة الثانية غذيت علي العليقة الأساسية مضافا لها 5مل زيت بذور اليقطين لكل كجم علف، المجموعة الثالثة غذيت علي العليقة الأساسية مضافا لها 200 ملجم كبريتات نحاس لكل كجم علف، المجموعة الرابعة غذيت علي العليقة الأساسية مضافا لها 5 مل زيت بذور اليقطين مع 200 ملجم كبريتات نحاس لكل كيلو جرام علف.

وأظهرت النتائج أن الأرانب في المجموعة الثالثة والرابعة تفوقت فى الوزن النهائي للجسم ومتوسط الوزن المكتسب وكذلك الدليل الانتاجي بينما أظهروا استهلاك علف أقل وتحسن في الكفاءة الغذائية. كما أظهرت المعاملة بكبريتات النحاس بمفردها أفضل قيمة لمعامل هضم المادة العضوية مقارنة بباقى المجاميع التجريبية كما أظهرت نفس المعاملة تحسن معنوى فى معامل هضم المادة الجافة والبروتين الخام عند مقارنتها بالكنترول أو تلك التى عوملت بزيت بذور اليقطين بمفرده فى حين لم يكن هناك فروق معنوية عند مقارنتها بالمعاملة المضاف إليها زيت بذور اليقطين مع كبريتات النحاس. كان هناك تحسن فى صفات الذبيحة نتيجة المعاملات خاصة المعاملة بكل من زيت بذور اليقطين مع كبريتات النحاس مقارنة بالكنترول. وبالنسبة لقياسات بلازما الدم زاد معنويا مستوى البروتين الكلى والجلوبيولين والكولستيرول عالي الكثافة فى جميع المعاملات مقارنة بالكنترول فى حين لاحظنا إنخفاضا معنويا في الاسبرتات امينو ترانسفيريز والكرياتينين والدهون الثلاثية والكولستيرول منخفض الكثافة والكولستيرول منخفض الكثافة جدا نتيجة المعاملات المختلفة مقارنة بالكنترول. وكذلك هرمون النمو والهرمون المحفز لإفراز هرمونات الغدة الدرقية وكذا هرمونى الغدة الدرقية T_4, T_3 زادوا معنويا فى المجاميع المعاملة خصوصا الخليط بين زيت اليقطين وكبريتات النحاس مقارنة بالكنترول. جميع المعاملات أدت إلى تحسن معنوى فى كل من IgG , IgM وحالة مضادات الاكسدة مقارنة بالكنترول. بالنسبة للكفاءة الإقتصادية تميزت المجموعة المعاملة بكبريتات النحاس بمفردها يليها المجموعة الخليط بين زيت اليقطين وكبريتات النحاس فى حين أظهرت المعاملة بزيت اليقطين بمفرده تدنى فى الكفاءة الإقتصادية وكان معنويا عند مقارنتة بالكنترول.

التوصية : أظهر زيت اليقطين تحسنا فى الأداء الإنتاجى وخصائص الدم المختلفة، وكذلك النحاس، إلا أن النحاس كان له ميزة كبيرة فى تحسين الكفاءة الإقتصادية، خاصة عند إضافته بمفرده، على عكس زيت اليقطين الذى أظهر إنخفاضا فى الكفاءة الإقتصادية مقارنة بالكنترول.