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EFFECT OF HYDROLYSABLE TANNIN AND GRAPE SEED EXTRACT SUPPLEMENTATION ON GROWTH PERFORMANCE, ANTIOXIDATIVE STATUS AND IMMUNE RESPONSE OF GROWING RABBITS DURING SUMMER SEASON.

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ABSTRACT:A total number of 60 weaned male New Zealand White rabbits at 6 weeks of age with an average initial body weight 704 ± 20g were randomly divided into four groups, fifteen rabbits /each during the experimental period (May–June 2020). First group was fed the basal diet without any additive as a control group, while 2nd ,3rd and 4th groups were the fed basal diet with hydrolysable tannin as Silvafeed® ATX (HT, 1.5 g/kg diet), grape seed extract (GE, 0.5 g/kg diet) and a mixture of HT and GE (HT-GE), respectively.

The results show that compared with other groups, the HT-GE significantly recorded the best body weight gain and feed conversion ratio compared with control. Whereas, there were no significant treatment effect on carcass characteristics. Indigenous enzyme activity in term of catalase activity was significantly higher both in blood plasma and meat as HT-GE introduced to the diet, meanwhile all supplements form decreased significantly lipid peroxidation as MDA compared to the control group. Plasma total protein, globulin and Immunoglobulin (IgG and IgM) were significantly the highest in the group HT-GE. Plasma cholesterol, triglyceride and liver enzyme functions were decreased without any significant affected between treatments. The economical efficiency ratio also increased with the treatment groups, especially the mixture between HT and GE.

Supplementation HT and GE had a beneficial effect as natural anti-oxidant additives by protecting against oxidative stress factors resulting from rearing rabbits through summer months by maintain performance, antioxidative status and immunity functions statuses of the rabbits.

Keywords: Antioxidant; immunity; hydrolysable tannin; grape seed extract; stress; rabbits

INTRODUCTION

Weaning is a critical period of growth for rabbits, which occurs as a result of the change in the rabbit's feeding and environment. An increase in the oxidative state is observed in the plasma after weaning. However, it is not clear whether this oxidative stress is caused by the weaning itself or other biological processes related to environmental factors. As environmental temperatures rise in Egypt, farm animals, including rabbits, are subjected to heat stress, which reduces their animal productivity. It has become imperative to provide natural and safe nutritional supplements to mitigate the negative effects of heat stress (Dangi *et al*., 2016). Cellular oxidative stress induced by heat stress as the formation of reactive oxygen species occurs as a result of creation free radical (Tawfeek *et al*., 2014). The adverse effect of biological activities occurred by mass production of free radicals lead to biological damage to DNA, proteins and lipids then affect the growth performance and productivity of animal's (Xing *et al*., 2019).

So, the alleviation of negative effect of high- temperature by using natural antioxidants feed supplementation become popular to removing the mass free radical (Liu *et al*., 2010 and Li *et al*., 2018). Hydrolysable tannins (HT) appear to be potential antioxidants. The stable phenoxyl radicals are formed by combination between free radicals and the water-soluble polyphenols present in the HT. This formulation has strong antioxidant properties (Rice Evans *et al*., 1996). Dalle Zotte *et al*. (2012) recorded that no significant effects in the health status, growth performance, and carcass characteristics of the developing rabbits by adding chestnut hydrolysable tannins at 200, 400, or 600 g / 100 kg diet. On the other hand, Liu *et al*. (2011) found that rabbits fed 5 or 10 g of chestnuts tannin / kg diet under heat stress conditions (33 °C) had an increased in growth performance. They suggested that tannins could be used to increased activities of antioxidant enzymes through direct interaction with free radicals. Grape seed extract (GE) is rich in polyphenols, including procyanidins and proanthocyanidins, which act as powerful free radical scavengers (Christaki, 2012; and Hassan *et al*., 2014a). In the same context, Grape seed proanthocyanidin extract contain a lot of polyphenolic antioxidants, a naturally occurring of oligomeric proanthocyanidins, known as condensed tannins, which are found in a wide scale of fruit and vegetables (Hassan *et al*., 2014b; Hajati *et al*., 2015 and Mansouri *et al*., 2015). Therefore, it is considered to be a better source of antioxidants than grape skins, another by-product of grape processing (Hassan *et al*., 2014a). Extracts from grape seeds and pomace are used as natural antioxidants and had a positive effect on rabbit performance (Choi *et al*., 2010 and Hassan *et al*., 2014b).

So, this study aimed to investigate the influences of using natural additive as HT and GE on the growth performance, carcass characteristics, oxidative status and plasma metabolites in growing rabbits.

MATERIAL AND METHODS

The present study was carried out in rabbit research unit at Borg El Arab Research Station located in Alexandria governorate, Egypt, belongs to Animal Production Research Institute, Agricultural Research Center.

Experimental diets and managements

The experimental diets were pelleted and formulated to meet recommended nutrient requirements of growing rabbits according to Lebas, (2013). Ingredient and calculated chemical composition of the basal diet are presented in Table 1. Rabbits were housed individually in stainless steel cages (35 ×35 ×60 cm^3) provided with feeders and automatic nipple drinkers. Diet and water were offered *ad libitum*. All rabbits were kept under the same management, hygienic and environmental conditions.

During the experimental period (May−June 2020), in the rabbitry the temperatures, the relative humidity and the temperature humidity index ranged 33.7- 34.8 ^oC, 83.8-84.4 % and 33.6 – 35, respectively. That means, during the whole experimental period rabbits were under severe heat stress

The temperature- humidity index (THI) was calculated according to Marai *et al*., (2001) using the modified formula :

$$THI = db^oC - [(0.31-0.31RH)(db^oC-14.4)]$$

Where db^oC dry bulb temperature in Celsius and RH= relative humidity percentage/100. A value for THI below 27.8 was taken to signify an absence of heat stress, while a value in excess of 28.9 was considered to present severe heat stress.

Experimental design

Sixty (six week of age) New Zealand White male rabbits were divided randomly into four groups (n=15 each) with 704±20 g average live body weight. The treated groups were, a control (basal diet without any supplementation Table,1), the second one was supplemented by hydrolysable tannin at 1.5 g/kg diet. Hydrolysable tannins (HT) as Silvafeed® ATX composed of 85% polyphenols, such as vescalagin, castalagin, roburin, procyanidins, proanthocyanidins, catechins, epigallocatechins, quercetin and others, was provided by Silvateam, Italy **(**according to Abdel-Khalek *et al*., (2016). It was added to feed just before pelleting. The third group was supplemented with grape seed extract (GE) at 0.5 g/kg diet. The extract contains phenolic (gallic acid, protocatechuic acid, pyrogallol, chlorogenic acid, catechol, procyanidin, vanillic acid, catechin, cinnamic acid, salicylic acid, syringic acid, chrysin and ferulic acid) and flavonoids (quercetin and others) according to Hassan *et al*., (2016). The fourth group was supplemented with a mixture of HT and GE (HT-GE). Throughout the experimental period, body weight was determined every four weeks (at 6,10 and 14 weeks of age) and average body weight gain was calculated. During the whole experimental period, the feed intake was determined precisely and is given as grams per rabbit per week. Feed conversion ratio (FCR) was calculated as a ratio of gram of feed per gram of gain.

Preparation of grape (*Vitis vinifera*) seed extract

Grape seeds were obtained from Haraz Company, Cairo, Egypt. One kilogram of grape seeds was ground to a fine powder, and soaked in ethanol (80% v/v) at room temperature for 24 h in the dark. After centrifugation at 4500 rpm for 10 min, the residue was re-extracted twice with 80% ethanol as described above. The supernatants were pooled together, concentrated in a rotary evaporator, the dry extract yield being 145.15 g of residue, and then stored at −35°C until being used (Mossa *et al*., 2015).

Carcass traits

At the end of the experimental period (14 weeks old), five rabbits from each treatment were fasted for 12h, weighed and slaughtered for carcass characteristics and meat analysis determination. Carcass characteristics were measured according to Blasco and Ouhayoun,(1996).

Blood metabolites and antioxidant parameters

Blood samples (about 5ml from each rabbit) were collected from the marginal ear vein to determine blood plasma components. Plasma was separated by centrifugation at 3000 rpm for 15 min and stored at −20°C until analyzed. Quantitative colorimetric determination of total protein (TP, g/dl), and albumin (Alb,g/dl) were executed by using kits of Stanbio Laboratory Inc, procedure No. 0280. (San Antonio, Texas, USA). Globulin concentration (Glb, g/dl) was calculated by subtracting Alb values from TP values. Albumin/ Globulin ratio (A/G ratio) was calculated. Quantitative colorimetric determination of triglycerides (TG mg/dl) and cholesterol (mg/dl) were executed by using kits of Spinreact, S.A./S.A.U. Ctra. Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN. The quantitative immunological determination of immunoglobulin G, immunoglobulin A and immunoglobulin M in plasma on COBAS INTEGRA by Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, USA . Kits from Diamond for lab technology (Heliopolis, Cairo - Egypt) were used in determination of concentrations (mg/dl) of urea and creatinine as indicators for kidney functions. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as indicators for liver functions were determined calorimetrically using kits supplied by Q-Slap, ElQasar El Ainy St, Cairo - Egypt). All determinations were performed according to the procedures outlined by the respective manufacturers.

Malondialdehyde (MDA) and catalase (CAT) were calorimetrically determined using commercial kits (purchased from Bio-Diagnostic, Cairo, Egypt, according to the manufacturers' instructions).

Economical efficiency

To determine the economical efficiency of the experimental diets for body weight gain, the costs of feed required for producing one kg of body weight gain was calculated. The cost of the experimental diets was calculated according to the price of different ingredients prevailing at local market during the experimental period (May−June 2020). Economical efficiency was calculated as a ratio between the return of weight gain and the cost of consumed feed.

Statistical analysis

The differences between experimental groups were statistically analyzed using the general linear model procedures of SAS (2001), applying one-way analysis of variance (ANOVA). The significant differences between treatments means were detected by Duncan's multiple range test ($P \leq 0.05$) (Duncan, 1955). All results were analyzed using the following statistical model:

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

Where: Y_{ij}= the observation of ij; μ = Overall mean; T_i= Effects of i (treatments) and e_{ij}= Experimental random error.

RESULTS AND DISCUSSION

Growth performance:

Initial body weight (g), final body weight and average daily weight gain from weaning (6 weeks old) until 14 weeks of age for growing New Zealand White rabbits, as affected by hydrolysable tannins (HT), grape seed extract (GE) supplementation, individually or in a mixture are presented in Table 2. All the treated groups were higher in the final body weight than the control one. The huge increase was in the group fed mixture (HT-GE) which was significantly higher than control group by 12.2% followed by group fed GE and HT diets groups by 7% and 5%, respectively as shown in Table 2. Also, the average daily weight gain significantly higher for group feed the mixture (HT-GE) than control through 10-14 and 6-14 weeks. The mixture (HT-GE) increased daily weight gain during the first 4 weeks of experimental period and the overall daily weight gain by 32% and 16%, respectively compared to control.The present finding was in agreement with other reports indicating that adding HT with different levels 0.5, 1 and 3% in rabbit's diet improved body weight gain (Zoccarato *et al*., 2008; Dalle Zotte and Cossu 2009 and Liu *et al*., 2012). It is clear that HT can protect intestinal mucosa against oxidative damage (Kermauner and Laurenčič, 2008), so increasing the efficiency of nitrogen retention subsequently increase body protein synthesis and deposition (Mroz *et al*., 2002). In this context, Schiavone *et al*., (2008) reported that birds fed with tannin had better final body weight when supplemented 0.2% chestnut wood in broiler diet. Additionally, the results are close to Hassan *et al*., (2014b, 2016) who reported that growing rabbits fed grape seed powder with 100, 200 and 300 mg GE/kg diet recorded significantly higher final body weight and daily weight gains through summer stress than the control group. So, a combination of hydrolysable tannins and grape seed extract is more beneficial for NZW rabbits than using them individually. This may be due to a synergistic effect of active components the two compounds.

Average daily feed intake (ADFI, g/day/rabbit) and feed conversions ratio (FCR) of the different treated groups are presented in Table 2. The ADFI of rabbits in GE group were significantly higher through 6-10 weeks and 6-14 weeks of fattening periods compared to control and HT groups. These results are in agreement with Zoccarato *et al*. (2008) who observed that rabbits fed a low protein amino acid supplement diet with 0.45% chestnut tannin had an improved average daily feed intake during the first 2 weeks post weaning. Conversely, Hassan *et al*. (2016) showed that no significant different in rabbit feed intake when supplemented 300 mg GE/kg diet.

Comparability to all treatments, the FCR of combination group was significantly improved ($p \leq 0.05$) through the all fattening periods. Also, Dalle Zotte and Cossu, (2009) recorded significantly improving in weight gain and FCR when adding 1 and 3% red quebracho tannins to rabbits diet. Similarly, Hassan *et al*., (2014b) found that supplemented GE improved significantly FCR in rabbit. This improvement may be due to the antioxidative effect of GE. These improvements in FCR were possibly due to the mode of action of phenolic compounds in GE and HT and their role in limiting peristaltic activity in digestive disorders, thereby preventing diarrhea (Kermauner and Laurenčič, 2008). Furthermore, the effect of HT in reducing intestinal movement might lead to better absorption of nutrients that enhanced body weight gain (Ismail *et al*., 2003) and Brenes *et al*., (2008) for broiler chickens and Hassan *et al*., (2014b and 2016) for growing rabbits.

Carcass characteristics:

The characteristics of carcass at the end of growth period are shown in Table 3. The results obtained are shown that adding HT, GE and HT-GE had no significant effect on all carcass parameters, However, the tendency in carcass characteristics percentage (dressing, Total edible part) were higher in growing rabbit treated with 0.5 g/kg GE followed by 1.5 g/kg HT then control group. While, edible parts percentage was higher in rabbit in HT group followed by GE group then in mixture of them. These results agree with Liu *et al*. (2009) and Tomažin *et al*. (2020) they reported that no variation on carcass weight and dressing percentage by adding HT (0%, 0.5%, and 1.0%) of a natural extract of chestnut wood (Silvafeed ENC) into the diet of rabbits or pigs. On the contrary, Hassan *et al*. (2016) reported that rabbits fed a diet containing GE led to an improvement in carcass weight, while total non-edible parts were significantly reduced compared to the control group.

Antioxidative status of plasma:

The effects of HT, GE and HT-GE on plasma antioxidant of rabbits are illustrated in Table 4. Plasma catalase activity were increased significantly ($p \leq 0.05$) in rabbits fed mixture HT-GE by 67% compared with control groups.

This increase in catalase activities could be attributed to the synergic effect between both HT and GE and their ability in enhancing the antioxidant system via increasing the activity of catalase, which is efficient in promoting the conversion of 6 million hydrogen peroxide to water and molecular oxygen/min (Sies, 2015). Antioxidant enzymes are the first line of defense to protect the organism from harmful peroxidation (Mao *et al*., 2014). The possible reason for these findings is that HT activates gene expression of antioxidant enzymes selectively by modulating redox-sensitive pathways by inhibiting lipid peroxidation and suppression of oxygen free radicals (Puiggròs, *et al*., 2005). With higher antioxidant enzymes concentrations, an improvement in the immune response and growth rate were found in the diet containing HT and GE.

This increased in catalase activity caused a significantly reduction in MDA by 21, 23 and 39% for HT, GE and HT-GE groups, respectively compared to control group. Therefore, the current study confirmed that the mentioned supplementations success to reduce the oxidative stress that occurs during the fattening periods through the hot months in Egypt (May and June). The results are in agreement with Almusawi *et al*. (2019) who showed that the supplementation of GE to poultry feeding led to a significant decrease in the MDA level and increased glutathione peroxidase enzymes, which may be due to phytochemicals present in grapes that have an antioxidant effect.

The polyphenol-rich GE may be an effective source of antioxidants in chicken diet, and the increase in the antioxidant effect of grape polyphenols in the excretory product indicates that a portion of the polyphenolate extract is capable of degradation by the action of intestinal bacteria (Brenes *et al*., 2008). The antioxidant action is the most important bioactivity of phenolic compounds which is for grape polyphenols twenty times more than vitamin E and fifty times more than vitamin C (Shi *et al*., 2003).

Also, Choi *et al*. (2010) who recorded decreasing in serum MDA levels when adding GE or grape peel powder to rabbits cholesterol diet plus GE or grape peel powder than in rabbits fed cholesterol alone. As shown in the study of Hassan *et al*. (2014b) plasma MDA slightly lower than control when adding GE (0.5- 1.5%) to rabbits diet.

Antioxidative status in meat:

As shown in Table 4, the GE and HT-GE groups had a significantly higher catalase activity in meat than the control group. In conjugation with the increased level of catalase, the MDA concentration significant decreased in treated groups compared to the control, these results confirmed the recorded results of antioxidant status in blood plasma. This results in agreement with Turcu *et al*., (2018) who showed that the inclusion of 2% grape seeds meal in broiler diets as a natural antioxidant inhibited the lipid degradation reactions of the meat. This decreased of MDA may be due to role of tannins against lipid peroxidation in meat which depending on their chemical structures, they act as scavengers directly interact with free radicals (Rojas and Brewer, 2008, Liu *et al*., 2011 and Dalle Zotte *et al*., 2018;) and forming relatively stable semiquinone radicals (Barbehenn and Constabel, 2011).

Chestnut tannins may stimulate the gene expression of the antioxidant enzyme, thus activating the endogenous antioxidant defense (Liu *et al*., 2011). Results from Liu *et al*., (2012) supports these hypotheses, as these authors found that tannin chestnut supplementation at 5 and 10 g / kg diet significantly reduced thiobarbituric acid-reactive substances (TBARS) values in meat of rabbits farmed under high environmental temperature. Hence, the ability of the used supplementation in enhancing the antioxidant system which in turn may enhance the fattening period of the growing rabbits during summer months (May and June).

Blood metabolites:

Table 5 clarifies that plasma TP levels were significantly ($p < 0.05$) in treated groups compared to the control. The Alb levels were not insignificantly affected by treatments. As for globulin, it increased significantly in the group fed HT-GE compared to the control group. It is known that globulins are carrier proteins for steroid and thyroid hormones and play a vital role in natural and acquired immunity to infection (Ganong, 2005 and Hassan *et al*., 2014b). The observed increase in globulins could be attributed to individual differences in the rabbits fed hydrolysable tannins and grape seed extract. High globulin level is a valuable index of immunological response and production of anti-bodies (Scanes, 2015 and Al-Yasiry *et al*., 2017).

Immunoglobulins:

From Table 5, it was obviously that, all immunoglobulin in terms of IgG and IgM were higher significantly in HT-GE group. Relieve stress weaning period by increasing the immunoglobulin occurs in rabbits, leading to improved immune function and help rabbits to develop the immune system Wang *et al*., (2011).

Immunoglobulin A is the major antibody involved in the mucosal immunity, which reduced intracellular pathogens through close association with a nonspecific innate defense system. Moreover, IgM is a second initial antibody produced in the initial stage of antibody response (Liu, *et al*. 2020a). Hence, in the present study there was a noticeable increase and improvement of immunoglobulin system of growing rabbits during the period from weaning to marketing age during summer. Also, these results assured the importance of HT and GE in enhancing the health of growing rabbits via enhancing both antioxidant and immune system. This results in agreement with Burke, (1994) who found that the animals feeding diet containing tannin had high immunity. Williams *et al*., (2017) found that diet supplemented with grape pomace in pig's diet cause increased in IgG, IgM and IgA, this polyphenol-rich diet in feeding monogastric animal cause increasing in granulocytes, and thus support for direct immunological effect of bioactive compounds within grape pomace. While the identify of these compounds cannot be definitively stated, the prime candidates were proanthocyanidins within grape pomace.

Liver and kidney function:

There were no significant differences in the plasma AST and ALT between the treated and control groups. The lowest value for AST was in the group fed HT-GE (Table 6). Meanwhile, the group of HT was the lowest one in ALT activity during the experimental period, and generally the treated groups were lower than the control in the activity of both AST and ALT which indicates that the treatment with HT and GE had no adverse effect on liver enzymes.

Plasma urea concentration decreased significantly ($P \leq 0.05$) in the group of HT compared to control groups. These results are in agreement with Liu *et al*., (2020b) who reported that the addition of HT to birds causing a decrease in serum urea. Moreover, Glantzounis *et al*. (2005) reported a decline in uric acid concentration, which has protective effects on oxidative stress in the body. Creatinine concentration decreased ($P<0.05$) in all treatment groups than control group. This result was in agreement with Abd El-Khalek *et al*. (2017) who reported that the GE added in the rabbit's diet reduced the level of creatinine in the plasma. From here it could be noticed that the substance used as feed additives had no adverse effect on liver functions (as measured by AST and ALT) and kidney functions (as measured by creatinine and urea levels).

Plasma lipid profile:

Cholesterol levels decreased insignificantly in rabbit's fed with HT and GE individual or in mixed form. These results were in agreement with Gai *et al*. (2010) and Hagerman *et al*. (1998) who found that diets high in tannins could reduce aortic malaldehyde and cholesterol hydroperoxides in rabbits. Decreased total cholesterol in plasma may be due to the role of GE in the strong binding to glycodoxycholic acid, while it is slightly bounds to taurocholic acid and taurodeoxycholic acid, indicating that GE may increase the excretion of fecal bile acid, resulting in a reduction of plasma cholesterol compared to a control group (Adisakwattana *et al*., 2010). Furthermore, the triglyceride level was decreased insignificantly by adding hydrolysable tannins and grape seed extract, but this effect was not significant. These results were in agreement with Akbari and Torki (2014) who suggest that a high concentration of antioxidants may reduce lipid oxidation and thus lower triglyceride levels in the blood.

Economical efficiency:

The effects of dietary supplementation of HT, GE and HT-GE on economical efficiency are shown in Table 7.

The results showed that the best economical efficiency and relative economic efficiency were recorded by rabbits in group fed HT-GE, which proved to be more economical and had higher net revenue than the other treatments included control group. This mixture of HT and GE achieved improvement performance in rabbits by promoting increase body weight and increased feed intake, then enhancing antioxidant capacity by improving dietary intake of antioxidants, which was reflected in economical efficiency (Dogan and Celik, 2012, and Hassan *et al.*, 2014b).

CONCLUSION

The results of this study showed that supplementation with combination of hydrolysable tannin (1.5g HT/kg diet) and grape seed extract (0.5g GE /kg diet) improved growth performance, and antioxidant status. This improvement is due to role of HT and GE, which are highly antioxidants, and the free radical scavenging effect in relation to the presence of a mixture of polyphenolic compounds, which are good antioxidant, especially for stressed rabbits. Additionally, this mixture achieved improvement in rabbit's performance via enhances body weight gain which resulted from increasing in feed intake, and enhance antioxidant system during summer considered critical for rabbits breeding. Generally, the two mentioned substrates are efficient in enhancing rabbits productive performance and physiological parameters.

Table (1): Feed ingredients and chemical composition of the basal diet on the dry matter basis.

Ingredients	Control
Alfalfa hay (12% CP)	25.00
Barley	12.00
Wheat bran	24.51
Yellow corn	12.50
Soybean meal (44% CP)	19.50
Molasses	3.00
Di calcium phosphate	2.00
Vit, & Min permix.[1]	0.30
S odium Chloride (NaCl)	0.30
Limestone	0.70
DL-Methionine	0.14
Anticoccidia (Diclazuril)	0.05
Total	100.00
Calculated chemical composition[2]	
Crude protein %	17.90
Digestible energy, kcal/kg	2543
Ether extract %	2.51
Crude fiber %	12.51
Calcium %	1.18
Total phosphorus %	0.86
Na %	0.16
Lysine %	0.95
Methionine %	0.41
Methionine+ Cyctine %	0.72

[1] Provided for each 1kg diet:- Vit. A, 6000IU; Vit. D_3, 900 IU; Vit. E, 40mg; Vit. B_1, 2mg; Vit. B_2, 4mg; Vit. B_6, 2mg; Vit. B_{12}, 10mg; Niacin, 50mg ; Pantothenic acid, 10mg; Biotin, 50mg; Folic acid, 3mg ; Choline, 250 mg ; Zn, 50mg; Mn, 8.5mg; Fe, 50 mg; Cu, 5mg; I, 0.2 mg; Se, 0.1mg and Co, 0.1mg.
[2] According the Egyptian Regional Center for Food and Feed (RCFF, 2001).

Table (2): Effect of dietary supplementation of tannin or grape seed extract (GE) or their combination on growth performance of New Zealand White rabbits.

Item	Experimental diet[1]				±S.E.M	Probability
	Control	HT	GE	HT-GE		
Average body weight (g/rabbits)						
Initial body weight(g)	708.9	683.1	711.1	715.3	35.4	NS
Final body weight (g)	1907^{b}	2001^{b}	2033^{ab}	2139^{a}	44.0	*
Average daily weight gain (g/day/rabbit)						
Weeks 6-10	25.1	27.3	28.3	27.7	1.24	NS
Weeks 10-14	17.7^{b}	18.5^{b}	19.0^{b}	23.4^{a}	1.25	**
Weeks 6-14	20.4^{b}	21.8^{ab}	22.4^{ab}	24.2^{a}	0.96	*
Average daily feed intake (g/day/rabbit)						
Weeks 6-10	76.1^{b}	76.9^{b}	83.7^{a}	80.3^{ab}	1.5	**
Weeks 10-14	105.٠	103.9	104.3	105.6	0.8	NS
Weeks 6-14	90.3^{b}	89.9^{b}	93.9^{a}	92.7^{ab}	0.9	*
Feed conversion ratio (g feed/g gain)						
Weeks 6-10	3.2	2.9	2.9	3.1	0.2	NS
Weeks 10-14	6.5	6.4	5.9	4.6	0.6	NS
Weeks 6-14	4.7^{a}	4.2^{ab}	4.3^{ab}	3.9^{b}	0.2	*

[1]HT = hydrolysable tannins (1.5g/kg diet), GE= grape seed extract (0.5g/kg diet) and HT-GE= mixture of HT+GE

$^{a-d}$ = Means in the same raw with different superscripts, differ significantly.

NS: Non-significant *: P≤ 0.05. **: P≤ 0.01. SEM: Standard errors of means.

Table (3): Effect of dietary supplementation of tannin or grape seed extract (GE) or their combination on carcass characteristics of New Zealand White rabbits.

Item	Experimental diet[1]				±S.E.M	Probability
	Control	HT	GE	HT-GE		
Pre-slaughter weight (g)	1965.0	2075.0	2040.0	1981.0	62.80	NS
Carcass weight (g)	1060.0	1121.7	1156.7	1091.7	29.90	NS
Dressing(%)	53.9	54.2	56.7	55.1	0.90	NS
Liver(%)	2.67	3.19	3.01	2.77	0.28	NS
Heart (%)	0.36	0.30	0.28	0.32	0.03	NS
Kidney (%)	0.68	0.78	0.75	0.77	0.07	NS
Spleen (g)	1.01	0.85	0.96	0.69	0.10	NS
Edible giblet%[2]	3.7	4.3	4.0	3.9	0.30	NS
Total edible parts%[3]	57.7	58.5	60.7	58.9	0.90	NS
Cecum weight(g)	124.6	103.3	89.3	121.3	14.00	NS
Cecum length (cm)	47.0	46.7	44.7	46.3	2.70	NS
Intestine length (cm)	259.3	260.0	255.0	269.0	14.60	NS

[1]HT = hydrolysable tannins (1.5g/kg diet), GE= grape seed extract (0.5g/kg diet) and HT-GE= mixture of HT+GE

[2] Edible Giblets %= (liver+ kidney + heart) / Pre-slaughter weight (g)*100

[3] Total edible parts %= (carcass wt. + edible giblets wt.) / Pre-slaughter weight (g)*100

NS: Non-significant SEM: Standard errors of means.

Table (4): Effect of dietary supplementations of tannin or grape seed extract or their combination on catalase and MDA in plasma and meat of New Zealand White rabbits.

Item	Experimental diet[1]				±S.E.M	Probability
	Control	HT	GE	HT-GE		
Antioxidant in plasma						
Catalase (U/L)	171.4[b]	244.6[ab]	242.9[ab]	285.8[a]	27.7	*
MDA(nmol/ml)	4.16[a]	3.30[b]	3.20[b]	2.53[b]	0.23	**
Antioxidant in meat						
Catalase (U/g)	100.0[c]	126.2[bc]	157.2[ab]	190.5[a]	10.9	**
MDA(nmol/g)	9.4[a]	8.5[b]	7.6[c]	7.6[c]	0.22	**

[1]HT = hydrolysable tannins (1.5g/kg diet), GE= grape seed extract (0.5g/kg diet) and HT-GE= mixture of HT+GE

[a-d] = Means in the same raw with different superscripts, differ significantly.

*: P≤ 0.05. **: P≤ 0.01. SEM: Standard errors of means.

Table (5): Effect of dietary supplementations of tannin or grape seed extract (GE) or their combination on the plasma proteins and immune response of New Zealand White rabbits.

Item	Experimental diet[1]				±S.E.M	Probability
	Control	HT	GE	HT-GE		
Plasma proteins						
Total Protein (g/dl)	4.1[b]	4.8[a]	4.9[a]	4.8[a]	0.18	*
Albumin (g/dl)	3.1	3.6	3.7	3.5	0.18	NS
Globulin (g/dl)	1.0[b]	1.2[ab]	1.1[ab]	1.3[a]	0.06	*
A/G ratio	3.1	3.0	3.4	2.7	0.24	NS
Immune indexes						
Immunoglobulin G (IgG, mg/dl)	226.3[b]	261.0[ab]	275.3[a]	281.0[a]	13.3	*
Immunoglobulin M (IgM, mg/dl)	14.3[b]	18.0[ab]	20.7[ab]	23.3[a]	2.0	*
Immunoglobulin A (IgA, mg/dl)	31.0	34.3	34.7	37.0	2.0	NS

[1]HT = hydrolysable tannins (1.5g/kg diet), GE= grape seed extract (0.5g/kg diet) and HT-GE= mixture of HT+GE

[a-d] = Means in the same raw with different superscripts, differ significantly.

NS: Non-significant *: P≤ 0.05. SEM: Standard errors of means.

Table (6): Effect of dietary supplementations of tannin or grape seed extract (GE) or their combination on the liver and kidney functions and plasma lipids of New Zealand White rabbits.

Item	Experimental diet[1]				±S.E.M	Probability
	Control	HT	GE	HT-GE		
Liver function						
AST (U/L).	33.3	17.7	24.3	15.3	5.2	NS
ALT (U/L).	39.0	25.7	34.3	30.7	8.7	NS
Kidney function:						
Urea (mg/dl).	46.0^{a}	23.7^{b}	40.7^{ab}	37.0^{ab}	6.2	*
Creatinine (mg/dl).	1.2^{a}	0.7^{b}	0.9^{b}	0.9^{b}	0.07	**
Plasma lipid profile :						
Cholesterol (mg/dl).	51.3	32.3	33.3	44.0	8.2	NS
Triglyceride (mg/dl).	117	104	114	92	12.08	NS

[1]HT = hydrolysable tannins (1.5g/kg diet), GE= grape seed extract (0.5g/kg diet) and HT-GE= mixture of HT+GE

[a-d] = Means in the same raw with different superscripts, differ significantly.

NS: Non-significant *: P≤ 0.05. **: P≤ 0.01. SEM: Standard errors of means.

Table (7): Effect of dietary supplementations of tannin or grape seed extract (GE) or their combination on economical efficiency of New Zealand white rabbits.

	Experimental diet[1]			
Item	**Control**	**HT**	**GE**	**HT-GE**
Average total weight gain/rabbit (kg)	1.20	1.32	1.32	1.42
Total revenue/rabbit(LE)[2]	59.9	65.9	66.1	71.2
Total feed intake/rabbit (kg)	5.06	5.03	5.26	5.19
Price of feeding/kg (LE)	4.50	4.56	4.52	4.59
Total cost of feed/ rabbit (LE)	22.8	23.0	23.8	23.8
Net revenue/ rabbit(LE)[3]	37.2	43.0	42.3	47.4
Economical efficiency[4]	1.63	1.87	1.78	1.99
Relative economical efficiency	100	115	109	122

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الملخص العربى

تأثير استخدام التانينات المتحلله و مستخلص بذور العنب على الأداء الإنتاجي وخصائص الذبيحة والحالة المضادة للأكسدة والاستجابة المناعية للأرانب النامية خلال فصل الصيف.

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تم تقسيم إجمالي عدد ٦٠ من ذكور الأرانب النيوزيلندية البيضاء المفطومة (عمر ٦ أسابيع بمتوسط وزن أولي للجسم ٧٠٤ ± ٢٠ جم) بشكل عشوائي إلى أربع معاملات، خمسة عشرة أرنب / لكل معاملة خلال شهور الصيف (مايو -يونيو ٢٠٢٠). المجموعة الأولى غذيت على العليقة الأساسية بدون أي مواد مضافة كمجموعة مقارنة، بينما غذيت المجموعة الثانية والثالثة والرابعة على العليقة الاساسية مضاف اليها التانينات المتحلله بالماء Silvafeed® ATX (HT، ١.٥ جم / كجم علف)، ومستخلص بذور العنب (GE، ٠.٥ جم / كجم علف) والخليط بين كل من HT وGE و (HT-GE) علي التوالي.

أظهرت النتائج، بالمقارنة مع المجموعات الأخرى، أن المجموعة الرابعة الخليط بين التانينات المتحلله بالماء ومستخلص بذور العنب (HT-GE) كان أفضل معنويا في وزن الجسم المكتسب ومعدل التحويل الغذائي. بينما لا يوجد تأثير معنوي للمواد المضافة للعلف على خصائص الذبيحة. كان نشاط إنزيم الكتاليز catalase أعلى بشكل ملحوظ في كل من بلازما الدم و اللحم في المجموعة المضاف لها خليط HT-GE إلى العليقة الاساسية بينما سجلت جميع جميع المعاملات المضاف لها الإضافات إنخفاضا معنويا في قيمة المالونالدهيد مقارنةً بمجموعة الكنترول. أدي إضافة خليط التانينات المتحلله مع مستخلص بذور العنب الي زيادة معنوية لكلا من: البروتين الكلي ،الجلوبيولين والجلوبولين المناعي(IgG و IgM). بينما انخفض الكوليسترول والدهون الثلاثية و إنزيمات

وظائف الكبد في البلازما في جميع المجموعات المعالجة بالإضافات بدون أي تأثير معنوي. كما زادت الكفاءة الاقتصادية النسبية لكل المجموعات المعالجة بالإضافات خاصة مجموعة الخليط (HT-GE). نستخلص من هذه الدراسة أن إضافة كلا من التانينات المتحلله أو بذور العنب أو خليطهما كإضافات مضادة للأكسدة طبيعية لهم تأثيرات مفيدة ضد عوامل الإجهاد التأكسدي الناتجة عن تربية الأرانب في أشهر الصيف من خلال الحفاظ على الأداء الإنتاجي والحالة المضادة للأكسدة و حالة الوظائف المناعية للارانب.