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PHYSIOLOGICAL AND PRODUCTIVE RESPONSES OF PREGNANT RABBITS TO DIETARY HYDROLYSABLE TANNINS AND GRAPE SEEDS EXTRACTSUPPLEMENTATION

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SUMMARY

The current study was undertaken to evaluate the physiological responses and productive performance of rabbit does as impacted by hydrolysable tannins (HT) and/or grape seeds extract (GSE) supplementation. A total number of 36 pregnant NZW rabbit does were used. They divided into four equal groups. The first group (G1) fed the basal diet without any supplementation and served as a control group. The other three groups were fed the same basal diet and supplied with HT at rate of 1.5 g/kg diet (G2), GSE at rate of 0.5 g/kg diet and a combination mixture of both supplements at the same rates (G4). Hormones, lipids and antioxidants capacity were quantified at days 14, 21 and at kindling day. Does performance was determined at mating, peak of lactation and weaning age. Litters performance was recorded at days of birth, 7, 14, 21 and weaning age. The present results revealed that, the highest levels of P4 and E2 were observed in G1 at day 14 and in G4 at day 21. Meanwhile, the lowest values of both hormones were noticed in G4 at kindling day. Does in G3 showed the lowest values of both TG and TC compared to the other treatments. All treatments were succeeded in improving the antioxidant capacity than that of G1, by lowering MDA values and increasing GPx activities. Daily milk yield increased in G4 than the other groups. There were no significant differences in does BW among all groups at different ages. Values of litters size and weight were lowest in G3 and highest in in G4 during the whole experimental period. In conclusion, the present results proved that all treatments were succeeded in improving both physiological responses and productive performance of pregnant does. Supplementing does with both HT and GSE together was the best and beneficial treatment followed by HT compared to control group.

Keywords: Pregnant rabbits, Hydrolysable tannins, Grape seeds extract, Physiological responses, Does and litters performance

INTRODUCTION

Rabbits are short cycle animals that can contribute significantly to the increasing demand of animal protein and can surmount the shortages in meat production due to their varied and excellent features. Therefore, rabbit farming is considered a vital industry in developing countries such as Egypt. From the economic point of view, the profitability of meat production is how much can the animal convert plant protein to meat, and rabbits can achieve this point of view by converting about 20% of the protein they eat to edible meat (Lebas *et al*., 1997). In addition, their meat is characterized by low fat and cholesterol contents and higher protein percentages (Ashour and Abdel-Rahman, 2019). Besides to their rolein solving the unemployment problem (Hamed *et al*., 2013).

For several years, antibiotics had been used to improve animal productivity by protecting the digestive tract from bacteria. However, several countries have been recommended the restriction of antibiotics in animal feeds. Due to the concerns about antibiotic residues in animal tissues, besides to the promotion of bacteria resistance to antibiotics. This leads to conduct excessive researches to find natural alternatives that promote animal health and production, among them, tannins in plants (Xu *et al*., 2023). Tannins were studied deeply, and many studies reported their positive role in enhancing animal performance. Elizondo *et al*. (2010) confirmed that tannins have a strong effect on animal intestine protozoa, viruses, and bacteria. Besides that, Mancini *et al.* (2019) mentioned that tannins are considered strong antioxidant that can improve animal health and productivity.

Another feed additive that has a powerful as an antioxidant and achieved positive results in improving rabbits' performance, which is grape seed extract. The extract of grape seeds isa promising antioxidant for dietary supplements. The extract also contains polyphenol compounds that have shown significant anti-inflammation effects on animals and human. In addition to the contributive molecules flavanols and procyanidins (oligomeric flavonoids), as reported by Hassan *et al*. (2014). Moreover, the antioxidant activity of grape is much more powerful in the extract of grape seeds than its grape skin (Hassan *et al*., 2014).

Many studies have been tested hydrolysable tannins and grape seeds extract and recorded their excellent effect on growing rabbits' performance. Such as Hassan *et al* (2014 and 2016), Manicini *et al.*

(2019) and Abd El-Azeem *et al.* (2021). However, testing these additives on pregnant rabbits have not been applied yet as far as we know.

Accordingly, the present study aims to examine and evaluate the impacts of dietary hydrolysable tannins and grape seeds extract either alone or in combination on physiological responses and productive performance of the NZW pregnant does and their litters during the breeding season of rabbits in Egypt.

MATERIALS AND METHODS

The current study has been applied in the Rabbits Research Unit in Borg El-Arab station, Alexandria governorate, which belongs to Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The experimental field of work was lasted for three consective months started from the beginning of October to the end of December.

Experimental animals:

Thirty-six of pregnant New Zealand White (NZW) rabbit does in their third parity have been used in this study. Their age about 8 months on average, with initial body weight (BW) of 3198.4±6.40 g. All does were kept individually in clean cages and having the same managerial and hygienic conditions. Does were healthy and no abnormalities were monitored. They were randomly divided into four equal groups (nine each). The first group (G1) served as the control one that received the basal diet based on the recommendations of NRC (1977) that cover rabbit requirements (Table, 1). The other three groups fed the same basal diet and supplemented with hydrolysable tannins (HT) at a rate of 1.5 g / kg diet (G2), as recommended by Abdel-Khalik *et al*. (2016), grape seeds extract (GSE) at a rate of 0.5g/kg diet (G3), based on Hassan *et al*. (2016) and a combination mixture of both additives (HT+GSE), respectively, at the same rates (G4).

The HT contained 85% polyphenols as outlined by the manufacturer was pushased from Silver feeds® ATX, Italy. While, the GSE was obtained from the National Research Center, Dokki, Giza, Egypt. As described by the manufacturer preparation of GSE have done via grinding the seeds of grape to get fine powder. Then soaking in ethanol alcohol (80%) and kept in a dark room for 24 h. After that, the preserved powder in alcohol was centrifuged for 10 minutes at 4500 rpm to take the residual and extracted twice in ethanol alcohol (80%). This extract contained phenolic compounds such as garlic acid, catechin, procyanidin. Addition of these additives was started before the does get inseminated for two weeks (as adaptation period) and continued until they does were born their kits and reashing to the weaning age at 35 days of these kits. No abortion cases or any abnormalities (such as getting infected with any diseases) have been noticed during the whole experimental period.

Table 1. Feed ingredients and chemical analysis of the basal diet (% dry matter basis) provided to the pregnant rabbit does during the experimental period

Feed ingredients	(%)	Chemical analysis (% DM basis)	
Soybean meal (44% CP)	8.50	Dry matter (DM)	89.00
Barley	30.0	Organic matter (OM)	90.88
Yellow corn	9.50		
Wheat bran	23.0	Crud protein (CP)	18.00
Clover hay	16.0	Crud fiber (CF)	10.60
Corn gluten (60%CP)	9.70		
Molasses	0.30		
Limestone	1.50	Ether extract (EE)	2.50
Di- calcium phosphate	0.50	Nitrogen free extract (NFE)	59.78
DL-Methionine	0.20	Ash	9.12
Sodium chloride	0.30	Lysine	0.98
Vit. -Min. premix[a]	0.30	Methionine+ cysteine	0.70
Anti-coccidiosis	0.10	Calcium	0.95
Anti-Fungi	0.10	Phosphorus	0.64
Total	100		

a=Each 1 kg contains: 12000 IU vit.A; 2200 IU vit. D3; 13.4 mg vit. E (determined); 2.0 mg vit. K3; 1.0 mg vit. B1; 4.0 mg vit. B2; 1.5 mg vit. B6; 0.0010 mg vit. B12; 6.7 mg vit. pantothenic; 6.67 mg vit. B5; 0.07 mg B8; 1.67 mg B9; 400 mg choline chloride; 133.4 mg Mg; 25.0 mg Fe; 22.3 mg Zn; 10.0 mg Mn;1 .67 mg Cu; 0.25 mg I and 0.033 mg Se

Blood sampling and analyses:

About 3ml of blood were collected three times at days, 14 and 21 of gestation period and at the kindling day from the marginal ear vein in heparinized test tubes. The samples were centrifuged at 3000 rpm for 20 minutes to get pure blood plasma and kept frozen at –20ºC until applying hormonal, lipids, and antioxidants analyses. The analyzed hormones were; progesterone (P4, ng/ml) and estrogen (E2, pg/ml). Hormones were quantified by Fluorescence Immunoassay Rapid Quantitative test using commercial kits and an FIA meter (Finecare

FIA meter plus), supplied from Guangzhou Wondfo Biotech co., China. The supplied kits from Bio-Diagnostic Company, Dokki, Giza, Egypt were used to determine both lipids parameters, triglycerides (TG, mg/dl) and total cholesterol (TC, mg/dl). In addition,kits from the same previous company were used to determine both of malondialdehyde (MDA, nmol/ml) and glutathione peroxidase (GPx, U/ml).These plasma parameters were quantified colorimetrically. All analyses procedures were executed according to the manufacturer pamphlets.

Does performance:

All experimental rabbit does were lasted in lactation for four continously weeks. Their daily milk production (DMY, g/day) was calculated based on the difference between their kits' body weight before and after suckling. Average of total milk production (TMY, g/d) during the whole lactation weeks was also calculated. Additionally, rabbit does body weight (BW, g) were weighed at; mating day, the peak (at the third week of lactation) of lactation, and weaning age of their kits. The amount of consumed feed (feed intake, FI, g/d) during the pregnancy and lactation periods was also calculated. Finally, litter size (LS) from each doe was recorded at days of birth, 7, 14, 21, and weaning age of litter.

Litter performance:

Litter mortality rate (MR, %) was also recorded during the entire experimental period. Their body weights (LBW) were recorded from kindling day reaching to their weaning age.

Statistical analysis:

The collected data were subjected to two-way analysis of variance to detect the effect of treatments (T) and the effect of gestation days (GD) and their interaction (T*GD) using the general linear model (GLM) procedure of SAS (1999).

The statistical model used was as follows:

$Y_{ijk} = \mu + T_i + GD_j + (T*GD)_{ij} + e_{ijk}$

Where: Y_{ijk}= the individual observation, μ = The overall mean, T_i= The fixed effect of the i^{th} treatments (i= 1,2,3,4), GD_j = the fixed effect of the j^{th} time (j=1, 2, 3), (T*GD) ij = Effect of interaction between i^{th} treatments and j^{th} the days of gestation (ij =1,…..12), e_{ijk}= Random error associated with the individual.

The collected data of dam weights, litter size and weight, and milk yield were subjected to a one-way analysis of variance to detect the effect of treatments. The statistical model used was as follows:

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

Where: Yij= the individual observation, μ = The overall mean, Ti= The fixed effect of the ith treatments (i= 1,2,3,4), and eij= Random error associated with the individual.

The differences among treatments, time, and interaction means were separated according to Duncan's Multiple Range Test (Duncan, 1955). The level of significance was set at 5% ($P<0.05$). A correlation between P4 and E2 levels and the measured lipids parameters was calculated.

Ethical Approval

All experimental procedures had been conducted according to ethical rules of Animal Production Research Institute (APRI).

RESULTS AND DISCUSSION

Physiological responses:

Progesteron and estrogen hormones:

Levels of P4 hormone were the highest in G1 compared to the other three treated groups (Table, 2). Therefore, the treatments of HT or GSE and their combination noticeably decreased P4 levels, especially in G4 followed by G3. These reductions were 53.9% in G4 and 44.3% in G3 ($P<0.05$) compared to that in G1. However, G2 had slightly and insignificantly lower level of P4 than that in G1. So, levels of P4 differed ($P<0.05$) in both G3 and G4 than those in both G1 and G2. Meanwhile, the difference between G1 and G2 was insignificant. Also, the difference between G3 and G4 was insignificant (Table, 2).

Owing to E2 hormone, its levels showed the opposite trend to that of P4 hormone. Its level was significantly ($P<0.05$) higher in G4 than that in G1 and G2 and insignificantly in G3 (Table, 2). It is obvious that, the synergetic effect of both HT and GSE together on reducing P4 and increasing E2 levels was more effective than that of HT alone and comparable with the treatment of GSE alone (Table, 2).

Concerning to the effect of gestation days, regardless of the treatments, P4 level decreased greatly and significantly ($P<0.05$) with the advancement of pregnancy period (Table,2). It is interesting to notice that, its level on day 14 equal 2.2 times that on day 21 and 8.4 times that at kindling day. This enormous decrease in P4 level with pregnancy progress is well known and documented in many studies as discussed below. Regarding the E2 hormone, its level changed greatly and significantly ($P<0.05$) among the gestation days. It was the highest on day 21 and the lowest one at kindling day. Where, its level increased ($P<0.05$) by 31.7% from day 14 to day 21. Meanwhile, it dropped ($P<0.05$) obviously by 40% from day 21 to that at kindling day (Table,2)

The interaction between treatments and gestation days revealed that G1 was superior group in the P4 level at day 14 of pregnancy and statistically differed than G3 and G4. At day 21 of gestation, the declined trend in P4 concentration was observed with no significant ($P>0.05$) difference among groups. Moreover, on the day 21, G4 was the lowest group in

P4 values and G2 was the highest one than the other experimental groups. Reaching to kindling day, all treated groups recorded lowest level of P4 than that in G1.

Table 2. Effect of hydrolysable tannins (HT) and grape seed extract (GSE) on progesterone (P4) and estrogen (E2) hormones level during pregnancy period and kindling day in NZW rabbits

	P4 (ng/ml)				E2 (pg/ml)			
Effect of treatment (T)								
G1	5.08[a]				35.49[b]			
G2	4.86[a]				34.32[b]			
G3	2.83[b]				37.78[ab]			
G4	2.34[b]				38.76[a]			
S.E	0.32				1.39			
Effect of gestation days (GD)								
14	7.21[a]				37.30[b]			
21	3.26[b]				50.57[a]			
Kindling	0.86[c]				21.89[c]			
S.E	0.28				1.20			
Effect of interaction (T×GD)								
	G1	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
14	10.86[a]	10.33[a]	3.85[b]	3.79[b]	38.26[cd]	36.59[d]	33.53[de]	40.83[bcd]
21	3.18[b]	3.55[b]	3.72[b]	2.55[bc]	45.97[b]	44.71[bc]	53.16[a]	58.44[a]
Kindling	1.18[cd]	0.69[d]	0.91[cd]	0.67[d]	22.23[fg]	21.68[fg]	26.64[ef]	17.01[g]
S.E	0.55	0.55	0.55	0.55	2.40	2.40	2.40	2.40

[a, b,c,d]Means in the same column with different superscripts are significantly different (P<0.05), G1= control group; G2= HT (1.5g/kg diet), G3= GSE (0.5g/kg diet) and G4= HT+ GSE.

For E2, the interaction reflected that G4 followed by G1 recorded the higher levels of E2 at the day 14 of gestation. Then at day 21, G4 kept their higher E2 level and significantly differed thanG1 and G2. On kindling day, all groups showed declined trend in E2 values than its levels during pregnancy. It is clear that, the highest levels of P4 and E2 were observed in G1 on day 14 and in G4 on day 21, respectively. Meanwhile, the lowest values of both hormones were noticed in G4 at kindling day.

The trend of P4 and E2 during gestation and kindling days are in agreement with Ashour and Abdel-Rahman (2019) who found that, P4 levels were 3.16, 4.00 and 1.58 at days; 14, 21 and kindling day, respectively, which are slightly close to the present data. Also, the same trend was observed for E2 levels that are in close to their recorded data in their results. The previous authors pointed out to the importance of P4 and E2 hormones during pregnancy period. The P4 hormone has a vital role in maintaining pregnancy via its positive impact on implantation. In addition, in late pregnancy, P4 going to decline to allow theE2 level to increase in order to let prostaglandins synthesize and increase in preparation for kindling. Besides that, P4 is very important to the first wave of mammary gland differentiation that called lactogenesis 1, which starts in the mid of gestation (Neville *et al*., 2002).

Also, the present results are close to those of Kirate *et al*. (2015). Who found that levels of P4 were 9.9, 5.3, and 4.1 ng/ml. But, lower than the finding of Alfonso (2016) who found that the P4 level on day 14 of pregnant rabbits was 12 ng/ml.

To the best of our knowledge, no studies have been carried out to test the mentioned additives on the levels of P4 and E2 in pregnant rabbits. Manzoor *et al*. (2020) determined P4 and E2 levels in rats received HT during their estrus cycle. They found that HT increased P4 level with no significant effect on E2 concentration.

Blood lipids:

As seen in Table (3), values of TG were comparable to each other without any significant difference. The lower and the highest values of TG were obtained in does ofG3 and G4, respectively. Concentrations of TG in all groups are within the normal physiological range of 54.2 -68.1 mg/dl as reported by Verga (2002). Regarding the concentration of TC, its vale ranged between 74.1 – 86.5 mg/dl among all groups which fall within the normal range 11.6 – 116.7 mg/dl (Verga, 2002). It is worthy to note that, does in G3 that treated with GSE alone showed the lowest values of both TG and TC compared to the other two treatments, HT (G2) or HT+GSE (G4). Furthermore, TC were reduced markedly and significantly (P<0.05) in does of values of G3 than those in G1 that showed the highest values of TC. It is well known that the reduction of blood TC is more beneficial for animal health. This reduction in both TG and TC by supplementing pregnant does with GSE alone could be attributed to the efficiency and powerful capacity of GSE in reducing blood lipids.

With respect to the effect of gestation days (GD), there were no significant differences in TG values among the GD. Whereas, values of TC differed ($P<0.05$) at the day 14 than those in both days 21 and kindling day. It is pronounced that, both TG and TC values were greatest at the day 14 and the lowest at the day 21 of pregnancy (Table,3). Day 14 in considered the beginning of the second trimester of pregnancy in rabbits. This finding is in accordance with that of Amir and Fessler (2013) who stated that, cholesterol is reached to its peak at the second trimester of pregnancy, while TG reached at the third trimester.

Regardless of treatments, during pregnancy and kindling days, TG and TC were at their higher levels at the day 14 of pregnancy, which is considered the beginning of the second trimester of pregnancy. This is somewhat agree and disagreed with Amir and Fessler (2013) who stated that TC reaches to its peak in the second trimester of pregnancy, while TG reaches in the third trimester.

According to the present data, both of TG and TC were decremented with pregnancy advancement. This is in the line with those of Kahilo *et al*. (2003) who recorded a gradual decrease in TG and cholesterol concentrations with pregnancy progress. They attributed this decline to the elevation in sex hormones biosynthesis.

Table 3. Effect of hydrolysable tannins (HT) and grape seed extract (GSE) on triglycerides (TG) and total cholesterol (TC) levels during pregnancy period and kindling day in NZW rabbits

	TG (mg/dl)	TC (mg/dl)
Effect of treatment (T)		
G1	66.73	86.45^{a}
G2	70.52	78.52^{ab}
G3	63.64	74.06^{b}
G4	73.36	84.27^{ab}
S.E	3.96	3.50
Effect of gestation days (GD)		
14	70.77	88.34^{a}
21	66.86	76.86^{b}
Kindling	68.07	77.26^{b}
S.E	3.43	3.03

Effect of interaction (T×GD)

	TG G1	TG G2	TG G3	TG G4	TC G1	TC G2	TC G3	TC G4
14	63.88	79.42	63.24	76.53	92.91^{a}	88.92^{ab}	81.72^{ab}	89.83^{ab}
21	63.25	66.43	64.63	73.14	81.55^{ab}	75.34^{ab}	70.93^{b}	79.62^{ab}
Kindling	73.06	65.70	63.07	70.43	84.87^{ab}	71.30^{b}	69.52^{b}	83.34^{ab}
S.E	6.86	6.86	6.86	6.86	6.06	6.06	6.06	6.06

[a, b] Means in the same column with different superscripts are significantly different ($P<0.05$), G1= control group; G2= HT (1.5g/kg diet), G3= GSE (0.5g/kg diet) and G4= HT+ GSE.

The interaction between the two factors (effect of treatments and pregnancy days) revealed that the treated groups (G2 and G4) were generally higher in TG levels than those in G1 except at kindling day. The general trend of TG was a decreasing trend from day 14 of pregnancy reaching to the kindling in G2 and G4. Meanwhile, TG levels in G3 were almost equal at days 14 and kindling. However, G1 recorded the opposite trend through achieving a higher TG level (73.06 mg/dl) on the kindling day, not on the day 14 as found in the other groups. As for TC, the interaction between the two factors showed gradual decrease in TC levels with pregnancy progress in all treated groups that did not statistically differed. However, G1 was the higher group in TC values than those of other groups. But, G4 and G1 were close to each other in TC values. Indeed, through our electronic search, a few literatures were discussed and explained the relationship between the applied treatments and those of lipids parameters. Some of them were applied on growing rabbits, human and other animals such as rats, but not in pregnant rabbits. Adisakwattana *et al*. (2010) illustrated that GSE can be used in the therapeutic protocol for people suffering from hyperlipidemia and obesity. Due to its ability in reducing TG and TC as found in rats. Because GSE contains oligomeric procyanidins that causing inhibition in pancreatic lipase that suppresses TG absorption. In addition, the ability of GSE in reducing cholesterol is attributed to the inhibition of cholesterol micellization in the lumen, leading to decreasing cholesterol absorption.

In rabbits, Yamakoshi *et al.* (1999) found that when rabbits were fed with highly TC diets and supplied with GSE significant reduction in TG and TC levels in their blood and protected them from heart disease. Abd El-Azeem *et al*. 2021 also recorded a reduction in blood TG and TC in growing rabbits due to the use of HT and GSE in rabbit diets.

Moreover, a correlation between these two lipid parameters and previously measured steroid hormones (P4 and E2) was applied. This correlation reflected that, P4 was positively correlated with TC (+0.44) and TG (+0.13). While, E2 was correlated with TG by +0.26 and negatively with TC (-0.27). This is in agreement with Amir and Fessler (2013) who illustrated that E2 and TC have anantagonistic relationship during pregnancy period.

Referring to Tables (2) and (3), E2 hormone reached to its higher level (50.57pg/ml) on the day 21 of pregnancy. Which coincides with the reduction in TC concentration from 88.34 mg/dl to 76.86 mg/dl at days 14 and 21 of pregnancy, respectively. This is in the line with the theory of, cholesterol is an important precursor for steroid hormone synthesis.

It should be pointed to the vital role of cholesterol during pregnancy. Cholesterol is a lipid molecule that can affect cell membrane via reducing cell fluidity and controlling permeability (Rousseau-Ralliard *et al*., 2023). Besides that, during pregnancy cholesterol can cross the placenta to provide nutritional requirements for the fetus and positively affects fetal growth, especially during the third trimester of pregnancy, and the subsequent birth weight (Wild and Feingold, 2023). While, TG can not across the placenta, but its products free fatty acids (FFA) and ketones can pass through the placenta to provide energy requirements for the fetus (Rousseau-Ralliard *et al*., 2023). So, maintaining these lipid parameters during the pregnancy period via providing pregnant does their nutritional requirements is very important to maintain fetal growth during pregnancy and born with suitable birth weight.

Antioxidant capacity:

As shown in Table (4), the effect of treatments on MDA levels was insignificant since all values were almost equal. However, the treated groups had slightly and insignificantly lower values than that in control group (G1). Where, the greatest and the lowest concentrations were observed in G1 and G4, respectively. On the other side, GPx activities showed opposite trend to that of MDA values, where the treated groups had higher activities than that in G1. Activities of GPx in does of G4 were higher significantly ($P<0.05$) than those of G1 by 39% (Table, 4). Meanwhile, the differences between G1 and those of G2 and G3 were insignificant. As well established, MDA inversely related to GPx. In G1, values of MDA were the highest due to the lowest values of GPx. Whereas, the opposite trend was obtained in G4, values of MDA were the lowest due to the highest values of GPx (Table, 4). These findings ascertained that the combined effect (G4) was more obvious in enhancing the antioxidant capacity followed by G3 then G2 compared to G1. Therefore, the three treatments were succeeded in improving antioxidant capacity than that of control group. Our results aretottaly agree with Abd El-Azeem *et al*. (2021) who found that mixing HT with GSE is better in enhancing oxidative stress in growing rabbits.

Among GD, regardless of the treatments, at day 14 MDA showed the lowest values which coincided with the highest activity of GPx. Whilst, at day 21 MDA exhibited the greatest values which accompanied with the lowest activity of GPx (Table, 4). This inverse relation is similar to that obtained by the effect of treatments. Concentration of MDA significantly ($P<0.05$) increased with pregnancy progress from day 14 to day 21 by 30.7%, then declined insignificantly at kindling day, but still higher than that recorded at day 14. Activities of GPx showed insignificant differences throughout GD, values were close to each other. These activities were the highest at day 14 then slightly decreased at day 21 and increased little bit at kindling day but still lower than that at day 14 of pregnancy.

The interaction effect of both T and GD showed that the MDA concentration ranged between the lowest value of 23.3 nmol/ml (G1 at day 14) and the greatest one of 48.6 nmol/ml (G1 at day 21) compared to the other treated groups. At day 14, there were no significant differences among all experimental groups in MDA values. Whilst, at day 21, MDA values interestingly elevated in all experimental groups with significant ($P<0.05$) difference between G1 and G3 (Table, 4). Also, at the same day, G3 was the lowest group in MDA level in comparison with the other groups. Furthermore, does in this group (G3) increased gradually and insignificantly their MDA values with advancement of pregnancy. Actually, the amount of increased MDA levels in the experimental groups from day 14 to the day 21 of pregnancy were; 108% in G1, 6% in G2, 3.6% in G3, and 23% in G4. The elevation in MDA is attributed to the decremented activity of GPx during pregnancy days in G1, G2 and G3, with fluctuation between increases and decreases from the day 14 to kindling day. Except G4 that showed a gradual increase in GPx activity during all pregnancy days. From day 21 to kindling day, MDA values decreased significantly ($P<0.05$) in G1 and insignificantly in G2 and G4. However, in G3 these values were increased insignificantly. Throughout GD, there were no significant differences in GPx activities among all experimental groups. Activities of GPx at day 21 showed the lowest (34.7 U/ml) in G1 and the highest one (58.4 U/ml) in G4 (Table, 4). Among GD, GPx activities were higher in G1, G2 and G3 and lower in G4 at day 14 compared to the other days of pregnancy. In contrast, at day 21 the opposite trend was observed. Therefore, it is clear that GPx activities decreased insignificantly in G1, G2 and G3 and increased in G4 from day 14 to day 21. Meanwhile, the opposite trend was seen from day 21 to kindling day.

Its well known that, pregnancy is a critical period that causes OS as confirmed by Krieing and Loch-Caruso (2001). When animals are exposed to any stressors, their body will produce free radicals that are rapidly removed in order to maintain their redox system. This happened by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). To work on elimination of free radicals and to reduce the products of lipid peroxidation (MDA), trying to prevent the damage in organs that occurred by MDA (Abdel-Khalek, 2013; Li CY Li *et al.*, 2017, and Garner *et al*., 2020).

Liang *et al.* (2022) confirmed that GSE is one of the antioxidants that can reduce OS in rabbits, especially during heat stress. This may be attributed to that GSE contains procyanidin, which is a type of flavonoid that has the ability to trap free radicals and limit lipid peroxidation. Moreover, this component (procyanidin) can inhibit the primary generator of free radicals, which is the xanthine enzyme (Hassan *et al*., 2016, and Garcia *et al*., 2002).

The present results agree with those of Abu Hafsa and Hassan (2022). They fed NZW rabbits with 50 g GSE/ Kg diet for 28 days. They found that, GSE succeeded in reducing OS induced by Lindane and attributed their results to the higher contents of polyphenolic compounds, Vit E, proteins, and anthocyanides in GSE. The anthocyanides have strong ability to free molecules of iron resulting in inhibiting iron-induced peroxidation of lipids.

For HT, Liu *et al*. (2011) noticed an improvement in antioxidant status in rabbits fed 5 and 10 g hydrolysable tannins/Kg diets. They cleared that; tannins can act directly on free radicals thus reducing negative impact of OS. In addition, Abd El-Azeem *et al.* (2021) stated that, HT are containing polyphenolic compounds that combine with free radicals to eliminate free radicals resulting from oxidative stress.

Table 4. Effect of hydrolysable tannins (HT) and grape seed extract (GSE) on antioxidant status during pregnancy period and kindling day in NZW rabbits

	MDA (nmol/ml)				GPx (U/ml)			
Effect of treatment (T)								
G1	34.99				38.05^{b}			
G2	33.24				44.09^{ab}			
G3	31.81				48.84^{ab}			
G4	31.22				52.90^{a}			
S.E	2.74				4.49			
Effect of gestation days (GD)								
14	28.53^{b}				48.77			
21	37.29^{a}				43.42			
Kindling	32.64^{ab}				45.72			
S.E	2.37				3.88			
Effect of interaction (T×GD)								
	G1	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
14	23.35^{b}	32.68^{b}	30.14^{b}	27.93^{b}	40.06	51.83	55.17	48.02
21	48.62^{a}	34.67^{ab}	31.23^{b}	34.63^{ab}	34.72	39.92	40.62	58.40
Kindling	33.00^{b}	32.40^{b}	34.07^{ab}	31.10^{b}	39.38	40.52	50.71	52.29
S.E	4.75	4.75	4.75	4.75	7.77	7.77	7.77	7.77

[a, b] Means in the same column with different superscripts are significantly different (P<0.05), G1= control group; G2= HT (1.5g/kg diet), G3= GSE (0.5g/kg diet) and G4= HT+ GSE.

Productive performance:

Milk yield:

It was obviously noticed that, supplementing does with both HT and GSE together increased the average of milk yield (DMY) in G4 than the other groups. These increases were significant with G1 and G3, whereas, insignificant with G2 in all weeks of lactation period (LP) except W1 (Table, 5). Also, the same trend was observed for the average of total milk yield (TMY). The average of TMY in G4 surpassed that of G1 by 27.5%. As well-established fact, all does reached their peak of lactation (PL) at the third week. This result is completely agree with Ashour *et al.* (2018). As seen that in Table (5), among all groups does increased their MY from W1 to W2. However, does in G3 decreased their MY than that of G2 during all weeks of lactation. These decreases were insignificant at W1 and significant (P<0.05) at the next three weeks of lactation. Does in G4 increased their MY during all lactation weeks. Therefore, supplementing does with GSE separately significantly (P<0.05) reduced DMY during W2, W3 and W4 and insignificantly at W1 compared to the other two treatments. The average DMY (g/day) of all does among all weeks ranged between the lowest value (91.1) which obtained in G1 at W1 to the highest value (161.9) which observed in G4 at W3. These values are in accordance of those of Ashour *et al.* (2018).

Table 5. Effect of hydrolysable tannins (HT) and grape seed extract (GSE) on average of daily milk yield (DMY, g/day) and average of total milk yield (ATMY) in NZW rabbits during the whole lactation period (4 weeks)

Groups	Weeks				ATMY
	1	2	3	4	
G1	100.8	104.7^{b}	140.6^{b}	91.11^{b}	109.3^{b}
G2	104.7	143.3^{a}	157.8^{a}	129.4^{a}	133.8^{a}
G3	96.38	116.1^{b}	139.1^{b}	97.5^{b}	112.3^{b}
G4	106.7	156.7^{a}	161.9^{a}	132.2^{a}	139.4^{a}
S.E	3.4	6.1	4.4	4.2	2.8

$^{a, b}$ Means in the same column with different superscripts are significantly different (P<0.05), G1= control group; G2= HT (1.5g/kg diet), G3= GSE (0.5g/kg diet) and G4= HT+ GSE.

To the best of my knowledge, research on the effect of HT or GSE on milk production in rabbit does has not been applied yet. It is well examined in ruminants such as cows and ewes. Mokni *et al*. (2016), who did not find any variations in ewes BW inspite of increasing their milk yield after feeding on 20% of GSE. In the same line, Herremans *et al.* (2020a and b) did not find any significant differences in milk production between the control group and the supplied group with HT in lactating cows. They pointed to the importance of supplying animals with feeds that naturally contain HT can help them to produce more milk than supplying with tannin extract Prapaiwong *et al.* (2023). Prapaiwong *et al*., 2023 clarified the ability of HT in reducing milk somatic cell counts (SCC) in dairy cows. Because HT has antibacterial activity through damaging lipid bilayer in bacteria cell membrane. As for GSE, our data are disagreeing with those of Mokni *et al*. (2016) who used 20% GSE in lactating ewes and found that their milk production increased by 300% after two months of feeding on it. They attributed their findings to the increase in energy supply that strongly correlated with milk production.

Feed intake:

During the whole pregnancy period (PP) and LP, G1 had the higher (P<0.05) quantity of the average feed intake (FI)than the other groups, particularly G4 that showed the lowest quantity (Table,6). The differences in FI quantities among the treated groups were not significant during both PP and LP, which differed significantly than those of G1. The most striking point that, the combined effect of both HT and GSE as in G4 increased (P<0.05) MY in spite of their lower FI in comparison with those in G1. The average of FI in does of G2 was the least compared to the other two treatments and at the same time produced reasonable quantity of milk that slightly and insignificantly differed than that of G4. This clearly indicate that, supplementing does with HT improved somewhat the feed efficiency (FE). Furthermore, G1 consumed more feed and produced less milk compared to G4 especially. This means that supplying does with mixture of both HT and GSE improved greatly FE. Thus, helping them to eat less and produce more. It could be concluded that, this mixture was more economical and efficient in increasing FE during rabbits rearing than using them separately (Table, 5 and 6).

Table 6. Effect of hydrolysable tannins (HT) and grape seed extract (GSE) on body weight and feed intake of New Zealand White rabbit does

	Doe weight at			Feed intake at	
Groups	**Mating**	**Peak of lactation**	**Weaning**	**pregnant**	**Lactation**
G1	3211.7	3180.6	3181.1	164.9^{a}	243.7^{a}
G2	3202.8	3198.3	3159.4	156.3^{b}	227.2^{b}
G3	3166.1	3137.3	3137.2	154.1^{b}	229.1^{b}
G4	3212.8	3182.7	3179.4	151.4^{b}	233.1^{b}
S. E	36.4	27.7	28.3	2.80	4.34

$^{a, b}$ Means in the same column with different superscripts are significantly different (P<0.05), G1= control group; G2= HT (1.5g/kg diet), G3= GSE (0.5g/kg diet) and G4= HT+ GSE.

***Body weight of does*:**

Values of does BW were close to each other, with no significant differences among them during different ages (Table, 6). These values of BWs decreased slightly from mating age (MA) to PL. This may be attributed to the combined effect of both stresses of pregnancy and lactation as confirmed by many studies. Whereas, from PL age to weaning age (WA), values of BW were almost equal. This may be due to the effect of lactation stress only. The does BW were little bit higher in G4 at WA than the other groups. This may be due to the synergestic effect of HT and GSE supplementation. In comparison among the treated groups at PL, the highest and lowest BWs were obtained in G2 and G3, respectively. Also, G3 showed the lowest values and G4 showed the highest values at both MA and WA. These differences may be due to the individual variations.

The present results disagree with Liu *et al*. (2011), who illustrated that HT caused an elevation in FI, thus increasing BW. Also, disagree with Hassan *et al*. (2014) who found that GSE increased FI and BW in rabbit bucks. While, Choi *et al.* (2010) supported our finding, they did not notice an improvement in growing rabbits BW in comparison with the control group.

As observed in Table (7), G3 showed the lowest values and G4 exhibited the highest values of LS during all ages from birth to weaning ages. The differences among all groups were not significant during he period from birth to 14 days of litters age. However, at day 21, LS of G4 differed significantly with that of G1 and G2. At WA there was significant difference between G4 and both G3 and G1, but insignificant with G2. It is clear that, G4 was the best group in this respect.

Litters size:

Table 7. Effect of hydrolysable tannins (HT) and grape seed extract (GSE) on litter size of NZW rabbit does

	Litter Size at:					
	Birth	**7**	**14**	**21**	**Weaning**	**Mortality rate%**
G1	6.0	5.9	5.9	5.7^{ab}	5.3^{b}	10.3
G2	6.2	6.2	6.1	6.0^{ab}	5.9^{ab}	4.67
G3	5.9	5.8	5.6	5.3^{b}	5.2^{b}	10.8
G4	7.0	6.8	6.8	6.8^{a}	6.7^{a}	4.19
S. E	0.43	0.42	0.42	0.37	0.37	3.04

[a, b] Means in the same column with different superscripts are significantly different (P<0.05), G1= control group; G2= HT (1.5g/kg diet), G3= GSE (0.5g/kg diet) and G4= HT+ GSE.

Mortality rates:

Mortality rate (MR, %) was the highest in G3 and lowest in G4 (P<0.05). it could be noticed that, G4 had the highest value of LS and the lowest MR during the entire experimental period compared to the other groups. From the economic point of view, this group which supplemented with HT and GSE considered the best treatment. This result was supported by the lower LS and higher MR in G3 than the other treatments and its values were nearly equal to those in G1. This means that, supplementing pregnant rabbits with GSE alone did not affect LS and MR. Nevertheless, G2 was slightly better than G1 and comparable with G4 in achieving higher LS and lower MR than those in G3. Therefore, it considered the best second treatment with HT alone was better than GSE. In the current study, MR ranged between 4.2 to 10.8% during the whole experimental period. These values were lower than that reported by Rashwan and Marai (2000). The authors mentioned that MR during pre-weaning age was estimated to be 16-20 %. The lower MR% in the present data may due to the suitable farm conditions. Such as weather conditions and providing good care and management for both does and their kits.

Litters body weight:

As seen in Table (8), during the whole experimental period, G3 had the lowest and G4 had the highest values of LBW. This may be attributed to the lowest MY in G3 and the highest MY in G4 (Table, 5). This higher MY in G4, enabled does for providing more milk to their kits especially in the first three weeks of lactation than the other groups. As mentioned by Ashour *et al.* (2018) kits are totally depending on dams' milk for 18 days of their age. Also, may be related to the TG andTC concentrations, which positively correlated with litter birth weight during gestation. Values of LBW in G4 differed significantly (P<0.05) than those in G1 and G3 and insignificantly with that of G2 during the whole experimental period except at day 21. Whereas, G4 differed significantly than the other three groups at day 21. The LBW increased by 6.7 (G1), 7.3 (G2), 6.6 (G3) and 7.3 (G4) times at weaning than that at birth. This increase was equal in both G2 and G4 and almost equal in G1 and G3. This is another confirmation that G4 is the best group followed by G2. The present results are in harmony with Abdel-Khalik *et al*. (2016). They provided 1.5 g HT/kg diet of growing kits, and they did not record any improvement in rabbit BW that fed on HT.

Table 8. Effect of hydrolysable tannins (HT) and grape seed extract (GSE) on litter body weight of NZW does

	Litter body weight at:				
	Birth	**7**	**14**	**21**	**Weaning**
G1	342.8^{b}	750.0^{b}	1190.0^{b}	1674.4^{b}	2301.7^{b}
G2	363.3^{ab}	821.7^{ab}	1326.1^{ab}	1817.8^{b}	2640.6^{ab}
G3	331.1^{b}	712.2^{b}	1097.2^{b}	1533.3^{b}	2195.0^{b}
G4	419.4^{a}	962.2^{a}	1520.6^{a}	2170.0^{a}	3067.8^{a}
S.E	21.6	52.2	85.0	112.6	167.0

[a,b] Means in the same column with different superscripts are significantly different (P<0.05). G1= control group; G2= HT (1.5g/kg diet), G3= GSE (0.5g/kg diet) and G4= HT + GSE

CONCLUSION

It could be concluded that, all treatments succeeded in improving the antioxidant capacity by lowering MDA and increasing GPx activity, particularly G4 compared to G1. The other physiological parameters were somewhat comparable to each other, except P4 concentration, especially in G3 and G4. Therefore, we recommend to use HT and/or GSE at levels not exceeded the present levels for monitoring P4 level and compeleting pregnancy safely in rabbits. In addition, supplementing pregnant does with a combination of both HT and GSE in G4 increased greatly E2 level, MY, LS, LBW and decreased MR compared to other groups.

Finally, our results confirmed that, addition of both HT plus GSE was the best and beneficial treatment, followed by HT alone.

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الإستجابة الفسيولوجية والإنتاجية للأرانب العشارلإضافة التانينات الذائبة ومستخلص بذور العنب في العليقة

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أجريت الدراسة الحالية لتقييم الاستجابات الفسيولوجية والأداء الإنتاجي للأرانب المغذاه على علف يحتوي على كلا من التانينات القابلة للتحلل المائي (HT) و/أو مستخلص بذور العنب (GSE). تم استخدام ٣٦ أرنبًا عشر من النيزيلندي الأبيض. وقسموا إلى أربع مجموعات متساوية. المجموعة الأولى (G1) تغذت العليقة الأساسية بدون أي إضافات وكانت بمثابة المجموعة الكنترول. وتم تغذية المجموعات الثلاث الأخرى بنفس النظام الغذائي الأساسي مع تزويدها بـ HT بمعدل ١,٥ جم / كجم من النظام الغذائي (G2) مستخلص بذور العنب بمعدل ٠,٥ جم / كجم من العليقة الأساسية وخليط مركب من كلا الإضافات المذكورة بنفس المعدلات (G4). تم قياس مستوي الهرمونات والدهون ومضادات الأكسدة في الأيام ١٤ و٢١ وفي يوم الولادة. وتم تقييم الأداء الإنتاجي لكلا من الأم والخلفات عند التلقيح، في مرحلة أعلي إنتاج للبن وعمر الفطام. تم تسجيل أداء المواليد عند أيام الولادة، ٧، ١٤، ٢١ وعمر الفطام.

أوضحت النتائج الحالية أن أعلى مستويات P4 وE2 لوحظت في G1 في اليوم ١٤ وفي G4 في اليوم ٢١. بينما لوحظت أقل قيم لكلا الهرمونين في G4 في يوم الولادة. أظهرت النتائج في G3 أقل قيم لكل من TG وTC مقارنة بالمعاملات الأخرى. نجحت جميع المعاملات في تحسين قدرة مضادات الأكسدة مقارنة بـ G1، عن طريق خفض قيم MDA وزيادة أنشطة GPx. زاد إنتاج اللبن اليومي في المجموعة الرابعة مقارنة بالمجموعات الأخرى. كانت قيم عدد ووزن الخلفات أقلها في G3 وأعلاها في G4 خلال الفترة التجريبية بأكملها.

أثبتت النتائج الحالية أن جميع المعاملات كانت ناجحة في تحسين الاستجابات الفسيولوجية والأداء الإنتاجي للأرانب العشار. وأن المكمل مع كل من HT وGSE معًا كانت أفضل معاملة يليهاHT مقارنة بالمجموعة الكنترول.