

## EFFECT OF ORAL ADMINISTRATION WITH GRAPE SEEDS EXTRACT ON GROWTH PERFORMANCE, CARCASS TRAITS AND BLOOD PARAMETERS OF GROWING RABBITS

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### SUMMARY

The current study was conducted to evaluate effect of oral grape seeds extract (GSE) at levels of 0, 75 and 125 mg/kg LBW daily for 21 days on growth performance, carcass traits and blood parameters of growing APRI rabbits. Total of fifteen growing APRI rabbit males having live body weight (LBW) of 618.67g and 8 weeks of age, were divided into three similar groups ( $n = 5$  in each) rabbits in the 1<sup>st</sup> group were given 3 ml sterile distilled water (Control, G1), while in the 2<sup>nd</sup> and 3<sup>rd</sup> groups were given 3 ml distilled water containing 75 (G2) or 125 mg/kg LBW (G3) grape seeds extract. All rabbits were fed commercial complete feed diet and kept under the same managerial and climatic conditions. Results show that, the average final body weight, total weight gain and daily body weight gain of rabbits in G3 were significantly higher ( $P<0.05$ ) (2180, 1564 and 27.93 g) than in G2 (2013, 1399 and 24.98 g) and control (G1) (1993, 1367 and 24.41g), respectively. Average daily feed intake was significant ( $P<0.05$ ) higher for rabbit with oral grape seeds extract at 12 mg than for control, but no significant affected between the two levels (G2 and G3). Results showed insignificant effect of oral grape seeds extract on average feed conversion ratio (gm feed/g gain). However, there was a tendency of reduction for rabbit treated with 125 mg/kg (G3) as compared to level 75 mg/kg (G2) and control group (G1). Results showed that oral grape seeds extract were insignificantly affected in all carcass parameters, however, the tendency in carcass characteristics values (weight after slaughter, weight with abdominal, giblets, empty, head, spleen, heart and kidney) higher in rabbits treated with 125mg/kg (G3) followed by treated with 75 mg/kg (G2) then control group (G1). Concentrations of total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL) and AST were significantly higher ( $P<0.05$ ) for growing rabbit in control group (G1) than both treatments (G2 and G3), while did not significant effect on concentration of total protein, albumin, globulin, A/G ratio, creatinine and activity of ALT enzyme. These findings suggest that oral of grape seeds extract with level of 125 mg/kg lead to improvement growth performance, health status and decreasing lipid contents in the body of APRI rabbits.

**Keywords:** Grape seeds extract, rabbits, growth performance, carcass trait, blood parameters.

### INTRODUCTION

Lipid peroxidation can be minimized by supplementation of antioxidant vitamins (Puthpongsiriporn *et al.*, 2001 and Franchini *et al.*, 2002) or natural substances that possess antioxidant potential (Sahin *et al.*, 2008 and Tuzcu *et al.*, 2008).

Antioxidants play an important role in protecting cells from reactive oxygen species by reducing free radicals and preventing the peroxidation of lipids (Grashorn, 2007 and Nanari *et al.*, 2004). Moreover, Benzie, (2003) reported that, antioxidant enzymes have the capacity to break down free-radical reactions using a chain reaction mechanism. The living organisms can synthesize some of these antioxidants, whereas others need to be provided by the diet (Strain and Benzie, 1999).

Flavonoids consist of the largest group of plant phenolics among the eight thousand naturally occurring phenolic compounds (Ruberto *et al.*, 2007). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. Studies have shown that flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Gonzalez- Paramas *et al.*, 2004; Yilmaz and Toledo, 2004; Ruberto *et al.*, 2007). Flavonoids having multiple hydroxyl groups, also act as pro-oxidants (Heim *et al.*, 2002; Miguel, 2010). The proposed mechanism of action for flavonoids is conversion of hydroxyl group into pro-oxidant when oxidized by

reactive oxygen species (ROS) present in inner cell membrane and able to oxidize lipids, proteins and DNA, thus may lead to late necrosis or apoptosis of damaged cells by eliminating potential mutants (Bakkali *et al.*, 2008). Many researchers conducted studies on medicinal plants and forages having polyphenolic compounds as an alternative strategy to treat coccidiosis. In a study on anticoccidial effect for grape seeds proanthocyanidine extract (natural polyphenolic antioxidant) against *E. tenella* infection, the significant reduction in mortality and improved bird performance was observed (Wang *et al.*, 2008). Proanthocyanidins are multifunction and powerful antioxidants having free radical scavenging activity (Cos *et al.*, 2003). Furthermore, proanthocyanidins are potent inhibitor of the proinflammatory cytokine and chemokine responses induced by lipopolysaccharides (Bodet *et al.*, 2006). Xanthohumol (XN), a prenylated flavonoid, obtained from the hops plant, has been reported to have anticoccidial activity against different *Eimeria* species in chickens (Allen, 2007).

The present study was carried out to study the effect of oral three levels of grape seeds extract (0.0, 75 and 125 mg/kg LBW) on growth performance, carcass traits and blood parameters of growing APRI rabbit bucks.

## **MATERIALS AND METHODS**

The experimental work was carried out in Rabbit Farm, Department of Animal Production, Faculty of Agriculture, Mansoura University, Mansoura, Egypt. During the period from August till November 2016.

### **Animals:**

A total number of 15 APRI line rabbits males (50 % Red Baladi bucks X 50 V line dose) were used in this study. The rabbits aged 8 weeks and weighted in average 618.67g. The rabbits were divided randomly equally on 3 groups, (n=5 in each group). Rabbits in the 1<sup>st</sup> group were given 3 ml sterile distilled water (Control, G1), while in the 2<sup>nd</sup> and 3<sup>rd</sup> groups were given 3 ml distilled water contained 75 (G2) or 125 mg/kg LBW (G3) grape seeds extract, respectively for 21 days as experimental period.

The requirement of the experimental diet and grape seeds extract for each weak were prepared according to the live body weight of rabbits weakly. The rabbits were fed *ad-libitum* on a commercial pelleted complete ration consist of barley, clover hay, wheat bran, yellow corn, soybean meal 44%, cotton seed meal, mineral and vitamin premix. The diet contained 2.3% fat, 16.5% crude protein, 13% crude fiber, 17% ash and 2740 Kcal digestible energy (DE)/kg on dry mater basis. Chemical composition of the experimental diet presented in Table (1).

All rabbits were housed in individual galvanized wire batteries arranged in rows, and located in conventional building ventilated by electric fans. All cages of were equipped with feeders made of galvanized metal and has nipple for automatic drinking water.

### **Experimental procedures:**

Live body weight of rabbits and feed intake were recorded weekly, then body weight gain and feed conversion ratio were calculated weekly throughout the excremental period from 8 to 16 wk. of age.

### **Biochemical analysis:**

Blood samples were collected from three rabbits in each group at the end of treatment. The blood samples were taken from the ear vein of bucks. Blood was taken into vacotainer tubes, centrifuged at 3000 rpm for 20 minutes to separate blood serum using serological pipettes, then serum was carefully decanted into labeled tubes and stored at -20°C until analysis. Concentration of total protein (Gornall *et al.*, 1949), albumin (Doumas *et al.*, 1971) glucose (Trinder, 1969) Total closterol (Potman and Stroes, 1968), creatinine (Tabacco, 979), high density lipoprotein and low density lipoprotein (Friedewald *et al.*, 1972), triglyceride (Bucolo and david,1973 ) as well as activity of aspartate (AST) and alanine (ALT) transaminases (Reitman and Frankel, 1957). However, concentration of globulin was calculated by subtracting albumin from total protein concentration. Albumin/globulin ratio (A/G) ratio was calculated.

### **Carcass traits:**

At the end of experimental period, 3 rabbits from each group were randomly taken, fasted for 12. hr to determine carcass characteristics according to Steven *et al.* (1981).

**Table (1): Ingredients and chemical composition of the experimental diet.**

Item	%
Ingredients, %	
Barely grains	32.80
Clover hay	27.41
Wheat bran	17.10
Soybean meal (44% CP)	16.24
Molasses	3.00
Di calcium phosphate	2.00
Sodium Chloride (NaCl)	0.30
Vit.& min. Mix*	0.30
Lime stone	0.40
DL-Methionine	0.40
Anticoccidia ( <u>Diclazuril</u> )	0.05
Total	100
Composition (calculated <sup>1</sup> )	
OM, %	89.76
CP, %	17.26
CF, %	12.92
EE, %	2.16
NFE, %	57.42
Ash, %	10.24
DE, kcal/kg	2518.7
Calcium, %	1.13
Total phosphorus, %	0.80
Methionine, %	0.64
Lysine, %	0.86

\*Each 1.5 kg contains: Vit. A 10.000.000 IU, Vit.B<sub>1</sub> 1000mg, Vit.B<sub>2</sub> 5000mg, Vit.D<sub>3</sub> 2.000.000 IU, Vit E 10.000mg, Vit. K<sub>3</sub> 1000 mg, Pantothenic acid 10.000mg; Nicotinic acid, 30.000g; Vit. B<sub>6</sub> 15000mg; Vit. B<sub>12</sub> 10 mg, Folic acid 1.0g, Biotin 50 mg, Cu 4g, choline chloride 200mg, Mn 60g, Fe 30g, Co 0.1 g, Se 0.1 g, Zn 50 g, Iodine 0.3 g and Antioxidant 10.000mg.

<sup>1</sup>according to Feed composition for animal and poultry feed stuff used in Egypt (2001).

#### **Statistical Analysis:**

The obtained data were statistically analyzed according to one way complete design to study the effect of treatment using SAS (2004). However, the significant differences among treatment groups were tested using Duncan's Multiple Range Test (Duncan, 1955). And the statistical moles was:

$$Y_{ij} = U + A_i + e_{ij}$$

Where:

Y<sub>ij</sub> = Observed traits

U = Overall mean

A<sub>i</sub> = Experimental group 1-3 (1= G1, 2= G2 and 3=G3)

e<sub>ij</sub> = Random error

## **RESULTS AND DISCUSSION**

#### **Growth performance:**

Live body weights (LBW) of rabbit bucks at the initial and end of treatment period are presented in Table (2).

**Table (2): Effect of different oral grape seeds extract levels on growth performance of growing rabbits.**

Item	(G1) Control	Oral grape seeds extract level	
		(G2) 75 mg/kg	(G3) 125 mg/kg
Experimental period (day)	56	56	56
Initial body weight(gm)	626.0±25.02	614.0±12.99	616.0±21.1
Final body weight (gm)	1993±50.73 <sup>b</sup>	2013±46.73 <sup>b</sup>	2180±37.98 <sup>a</sup>
Total weight gain (gm)	1367.0±65.83 <sup>b</sup>	1399.0±41.93 <sup>b</sup>	1564.0±23.9 <sup>a</sup>
Daily body weight gain (gm)	24.41±1.18 <sup>b</sup>	24.98±0.75 <sup>b</sup>	27.93±0.43 <sup>a</sup>
Daily feed intake (gm)	77.6±2.23 <sup>b</sup>	80.81±1.71 <sup>ab</sup>	83.4±0.73 <sup>a</sup>
Feed conversion ratio (g feed/g gain)	3.18±0.19	3.23±0.05	2.99±0.05

a and b--- Means in the same row with different superscripts are significantly different ( $P<0.05$ ).

Analysis of variance revealed that LBW of rabbits was not affected significantly at initial of treatment, while at the end of oral grape seed extract treatment, the average final body weight, total weight gain and daily body weight gain of G3 (with level of 125 mg/kg) were significantly higher ( $P>0.05$ ) (2180, 1564 and 27.93 g) than group (G2) with level of 75 mg/kg (2013, 1399 and 24.98 g) and control (G1) (1993, 1367 and 24.41g), respectively (Table 2).

Average daily feed intake for rabbit bucks was significant affected ( $P<0.05$ ) by oral grape seeds extract, showed higher values for rabbit bucks treated with both levels than for control, but no significant affected between the two levels (G2 and G3).

Results showed insignificant effect for oral grape seeds extract on feed conversion ratio. However, there was a tendency of reduction in rabbits treated with level 125gm/kg (G3) as compared to level of 75 gm/kg (G2) and control group (G1) during experimental periods.

Increasing of GSE level in the chicken diets did not change the growth performance, feed consumption and feed conversion ratio at 21 and 42 days of age compared with those fed control diet, Brenes *et al.* (2010)

#### Carcass characteristics:

Carcass characteristics at the end experimental period are presented in Table (3). The obtained results showed that oral grape seeds extract were insignificantly effected in all carcass parameters, However, the tendency in carcass characteristics values (weight after slaughter, weight with abdominal, giblets, empty, head, spleen, heart and kidney) higher in rabbit bucks treated with 125gm/kg (G3) followed by 75 gm/kg (G2) then control group (G1). While, liver was higher in rabbit bucks in G3 followed by control group then in group 2 (G2). But, the tendency in carcass characteristics (lung, bile, shoulder fat and abdominal fat) higher in rabbit bucks treated with 75 gm/kg (G2) followed by 125gm/kg (G3) then control group (G1).

The graded concentrations of GSE did not affect the relative organ weights (pancreas, liver, liver fat and abdominal fat), except for spleen which was increased up to 14% at 42 days of birds age fed the highest concentrations of GSE compared with those fed control diet (Brenes *et al.*, 2010).

Hassan, *et al.* (2016) reported that dietary GSE improved carcass weight, percentage of hot carcass, intestine and edible giblets, while total non-edible parts were reduced ( $P\leq0.05$ ) in comparison with the control group.

Regarding relative organ lengths, the inclusion of graded concentrations of GSE caused a significant decrease of jejunum (up to 18%; linear and quadratic effect), ileum (up to 19%; linear effect) and ceca (up to 9%; linear effect) at 21 days of age. However, at 42 days of age, an increase in the relative lengths of jejunum (6%), ileum (9%) and ceca (14%) was only observed in birds fed the highest GSE concentration in the diet (Brenes *et al.*, 2010).

#### Blood parameters:

The serum values of some blood constituents are shown in Table (4). The obtained values were within the normal range. Concentration of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) were significantly ( $P<0.05$ ) higher in control group (G1) than both treatments (G2 and G3). While concentration of high density lipoprotein (HDL) was slightly ( $P<0.05$ ) higher in group 2 than other

groups glucose and creatinine concentration ( $P<0.05$ ) decreased in both treatments (G2 and G3) than control group (G1).

**Table (3): Effect of different oral grape seeds extract levels on carcass characteristics for growing rabbits.**

Item	(G1) Control	Oral grape seeds extract level (G2) 75 mg/kg	(G3) 125 mg/kg
Live weight, g	1990.0±79.37	1963.33±48.42	2121.67±61.73
Weight after slaughter, g	1933.33±74.96	1915.0±50.33	2058.33±58.33
Weight with abdominal, g	1435.0±60.0	1446.67±52.63	1561.67±46.31
Giblets, g	416.67±23.51	378.33±19.22	433.33±36.09
Empty, g	896.67±54.87	998.33±72.59	1041.67±33.71
Head, g	118.33±4.41	120.0±2.89	128.33±1.67
Liver, g	64.47±2.24	55.83±2.86	66.433±2.19
Kidney, g	15.73±2.18	17.17±1.16	17.90±2.56
Lung, g	13.67±1.33	19.37±2.04	18.77±2.64
Bile, g	1.30±0.26	2.27±0.57	1.17±0.09
Spleen, g	1.13±0.19	3.07±0.94	4.77±1.96
Heart, g	6.17±0.20	8.07±1.20	12.60±3.08
Shoulder fat, g	3.23±0.42	4.70±0.91	2.47±1.44
Abdominal fat, g	15.57±5.29	16.0±3.28	5.10±2.89

**Table (4): Effect of different oral grape seeds extract levels on blood constituents for growing rabbits.**

Item	(G1) Control	Oral grape seed extract level (G2) 75 mg/kg	(G3) 125 mg/kg
ALT(IU/l)	26.33±5.11	30.33±5.39	33.33±3.1
AST(IU/l)	26.33±5.93 <sup>a</sup>	14.50±1.88 <sup>b</sup>	13.83±1.89 <sup>b</sup>
Total Protein (g/dl)	5.92±0.42	5.48±0.22	5.53±0.30
Albumin (g/dl)	2.61±0.25	2.87±0.104	2.91±0.14
Globulin (g/dl)	3.3±0.21	2.62±0.22	2.62±0.27
A/G ratio	79.05±5.67	114.44±12.06	118.76±17.93
Creatinine (mg/dl)	2.88±1.62	1.32±0.07	1.03±0.06
Total cholesterol (TC) (mg/dl)	67.17±1.35 <sup>a</sup>	52.17±3.36 <sup>b</sup>	50.17±2.07 <sup>b</sup>
Triglyceride (TG)	34.17±2.34 <sup>a</sup>	20.83±2.88 <sup>b</sup>	23.83±4.5 <sup>b</sup>
Glucose (mg/dl)	33.33±5.89	22.83±1.87	22.17±2.01
HDL (mg/dl)	34.0±5.16	37.33±1.93	35.83±1.14
LDL (mg/dl)	27.17±5.28 <sup>a</sup>	12.0±1.51 <sup>b</sup>	10.67±0.604 <sup>b</sup>

*a* and *b*-- Means in the same row with different superscripts are significantly different ( $P<0.05$ ).

Some of studies has clearly revealed that polyphenols, mainly catechins, have antioxidative properties by the inhibition of low density lipoproteins (LDL) oxidation (Simonetti *et al.*, 2002 and Shi *et al.*, 2003). Furthermore, epicatechin, also protects endothelial cells against oxidized LDL by scavenging free radicals and maintaining nitric oxide synthase (Steffen *et al.*, 2005).

Hassan, *et al.* (2016) found that plasma total protein, albumin and globulin were ( $P\leq 0.05$ ) increased in New Zealand white rabbits fed GSE at 200 and 300 mg/kg LBW. In contrast, all supplementation levels of GSE ( $P\leq 0.05$ ) reduced concentrations of total lipids, total cholesterol, triglycerides and low-density lipoproteins. Antioxidant enzymes of rabbits (superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase) and total antioxidant capacity in blood were ( $P\leq 0.05$ ) increased by fed GSE. However, malondialdehyde was ( $P\leq 0.05$ ) reduced with increasing GSE levels.

Analysis of variance reveled that the activity of AST enzyme was significantly ( $P<0.05$ ) lower in both treatments (G2 and G3) than control (G1) group (14.5, 13.83 and 26.33 IU/l), respectively. While the activity of ALT enzyme in opposite tendency, but not significant (Table 4).

The antioxidant compounds present in grape seeds have been identified as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives, flavan-3-ols (catechin and epicatechin), flavonols (quercetin and myricetin), and anthocyanidins (Caillet *et al.*, 2006).

Shi *et al.* (2003) reported that the antioxidant potential of grape seeds is twenty and fifty fold greater than vitamins E and C, respectively, arising from increased levels of polyphenols proanthocyanidins and oligomers of flavan-3-ol units, especially catechin and epicatechin present in GSE (Yilmaz and Toledo, 2004).

## **CONCLUSION**

These findings suggest that oral of grape seeds extract with level of 125 mg/kg lead to improvement growth performance, health status and decreasing lipid contents in the body of APRI rabbits.

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## تأثير تجريب مستخلص بذور العنبر على أداء النمو وخصائص الذبيحة ومقاييس الدم لذكور الأرانب النامية

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أجريت هذه الدراسة بغرض معرفة تأثير تجريب مستخلص بذور العنبر بمستويات ( صفر , 75 , 125 ملigram/ كجم ورن حي) لمدة 21 يوم على أداء النمو وخصائص الذبيحة ومقاييس الدم لذكور الأرانب النامية. استخدم 15 ذكر أرنب بمتوسط وزن 618.67 جم وعمر 8 أسابيع ، قسمت إلى ثلاثة مجاميع متماثلة (5 ذكور لكل مجموعة). المجموعة الأولى أعطيت 3 مليلتر ماء مُغطّر مُقمّ (كتنرول مجموعة 1)، بينما المجموعات الثانية والثالثة أعطيت 3 مليلتر ماء مقطّر يحتوى على 75 ملجم/ كيلوغرام وزن حي (مجموعة 2) أو 125 ملجم/ كيلوغرام وزن حي (مجموعة 3) من مستخلص بذور العنبر. كل الأرانب غذيت على علقة متكاملة تجارية وتحت نفس الظروف الجوية والرعاية. وكانت النتائج المتحصل عليها كما يلى:-

وجد أن متوسط وزن الجسم النهائي والزيادة الكلية والزيادة اليومية للمجموعة 3 (2180 , 1564 , 27.93 جم) أعلى بمعنىه ( $P<0.05$ ) عن المجموعة 2 (1993 , 1399 و 24.98 جم) وعن مجموعة الكتنرول (1367 و 24.41 جم) على التوالي.

وجد أن متوسط الغذاء المأكول للأرانب المعاملة اعلى بمعنىه ( $P<0.05$ ) عن مجموعة الكتنرول ولكن لم يكن هناك فروق معنوية بين المعاملات.

أظهرت النتائج عدم معنوية تأثير المعاملة بمستخلص بذور العنبر على متوسط التحويل الغذائي للأرانب (جم علبة/جم زيادة في الوزن). وعلى أية حال الاتجاه يميل إلى النقص مع الأرانب المعاملة بمستوى 125 ملagram مقارنة مع الأرانب المعاملة بمستوى 75 مليجرام والكتنرول.

أظهرت النتائج عدم معنوية تأثير المعاملة بمستخلص بذور العنبر على كل خصائص الذبيحة، بينما كانت قيم خصائص الذبيحة للأرانب المعاملة بمستوى 125 مليجرام الأعلى في الوزن بعد الذبح وبالأحساء، الأحساء والوزن فارغ، الرأس ، الطحال ، القلب والكلية) يليها الأرانب المعاملة بمستوى 75 مليجرام بيلها الكتنرول.

التركيز الكلى للكوليستيرول ، الكوليستيرول الأقل كثافة والجلسيريات للأرانب في مجموعة الكتنرول اعلى بمعنىه ( $P<0.05$ ) عن الأرانب في مجموعة 2 و 3 (المعاملة بمستخلص بذور العنبر)، بينما تركيز البروتين الكلى، الالبومين، الجلوبولين، نسبة الالبومين/الجلوبولين، الكرياتينين، نشاط إنزيم ALT، الجلوكوز والكتنرول الاعلى كثافة لم يتأثر معنويًا بالمعاملة.

نستخلص من هذه الدراسة أن تجربة ذكور أرانب الابرى بمستخلص بذور العنبر بمستوى 125 مليجرام/كجم وزن جسم أدى إلى تحسن في أداء النمو والحالة الصحية مع نقص اليبيادات في الجسم.