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Growth Performance, Nutrients Digestibility and some Blood Constituents in Growing New Zealand White Rabbits Fed Diets Supplemented with *Eucalyptus globules*

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ABSTRACT

This study was conducted to investigate the effects of supplementing the rabbit diets with *Eucalyptus globulus* dried leaves (EGL) or its aqueous extract (EGE) on growth performance, nutrients digestibility and blood constituents. A total number of 225 weaned New Zealand White rabbits, six weeks old, were used in this experiment. Treatment groups were ; a control diet (C), T^1 and T^2 were the control diet + 0.25 or 0.5% dried eucalyptus leaves, respectively, T^3 and T^4 were the control diet + 0.05 or 0.1% aqueous extract of eucalyptus leaves, respectively, The growth trail lasted for 8 weeks. At the end of the trial, digestibility nutrient trails were done and nutritive values were calculated, then rabbits were slaughtered and blood samples were obtained. The obtained results could be summarized as follows: Providing rabbit diets with EGL; EGA significantly ($P<0.01$) increased final live weight, body weight gain and, feed conversion ratio, especially at 0.25% EGL compared to the control. The best FCR were reported to the groups contain 0.25% EGL followed by 0.05% EGE. The digestibility coefficients for OM, CP and nutritive values in terms DCP and TDN of the experimental diets significantly increased with EGL and EGE feeding. Supplemented EGL or EGE to the diets significantly increased ($P<0.05$) values of hemoglobin concentration, RBC's, WBC's count and percentage of lymphocytes. Both EGL and EGE significantly increased blood total protein and albumin, also decreased triglycerides and total cholesterol and has no effect on creatinine and the activity of AST and ALT. It could suggested that using eucalyptus as feed additives for growing rabbits improve growth performance and digestibility, with no adverse effects on blood constituents.

Keywords: eucalyptus globules, rabbits, growth performance, digestibility, blood constituents

INTRODUCTION

The rabbit production is an important source of meat in Egypt. The rabbit farming provides additional income to small farmers which may be a source of their livelihood and increasing opportunity for employment to weaker section of rural community. In addition to high biological value amino acids of rabbit meat, it is high in protein, with low of cholesterol and sodium (Chakrabarti *et al*., 1999). Rabbits are very sensitive to enteric diseases especially when they are exposed to negative effects causing high losses such as weaning or heat stress (Shrivastava *et al*., 2012). Antibiotics were used to get rid of these problems and as growth promoter in animal feed, which improved health state and increased nutrient availability for rabbits, this lead to increasing growth performance (Pinheiro *et al*., 2004). However, the general intention to limit antibiotics in animal feed as growth promoter because of its side-effects, resistance and recent public perception about healthy food, besides, the ban on antibiotic growth stimulators in animal nutrition in the European Union, so new alternatives to antibiotics are needed (Marzo, 2001), such as herbal preparations (Abd El-Hady *et al*., 2013; Nosal *et al*., 2014 and N'Guessan *et al*., 2015).

Eucalyptus globules leaves are also known to contain bioactive products that showed anticariogenic (Ishnava *et al*., 2013), analgesic (Cimanga *et al*., 2002), antihistaminic and anti-inflammatory effects (Nagpal *et al*., 2010) and antioxidantive (Vratnica *et al*., 2011) activities. *Eucalyptus globules* leaves contain 1,8-cineole, eucalyptole and the other major components were alpha and beta piene, camphene, alpha-phellandren, P-cymene, crptone, alpha-terpineol, terpin 1-ene-4-ol, globulol and spathulenol (Chalchat, 1995).

Waly (2004) found that 0.4% dried Eucalyptus globulus leaves used as feed additives in broiler diets improved weight gain, feed conversion ratio, nutrients digestibility, sensory evaluation and economical efficiency. Also, Osman *et al.* (2007) concluded that 0.1% *Eucalyptus globulus* leaves improved performance per chicken. Silva *et al*., (2003) concluded that not water extracts of dried leaves of *Eucalyptus spp* traditionally used as analgesic, anti-inflammatory and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion. Sugimoto *et al*. (2005) found that the extracts of Eucalyptus globulus showed efficiency in preventing the oxidation process.

The aim of this trial was to test the effect of *Eucalyptus globulus* dried leaves or its aqueous extract growth performance, nutrients digestibility and blood constituents of growing weaned New Zealand White rabbits.

MATERIALS AND METHODS

Plant material

Eucalyptus leaves were air dried at room temperature in the shade for a few weeks to a final moisture content of 10.0%. Then, the dried samples were ground in a blender so that the particle size will be between 0.8-0.9 mm.

Preparation of aqueous extract

Weighting 100 gm of Eucalyptus globulus powdered leaves was infused in 500 ml hot water for 4 hours then filtered with Whattman filter paper. Extracts were kept in Deep freezer at - 4°C for 48 hours, then introduced in freeze dryer till completely dried. The residue was weighed and the yield percentage was determined (Mittal and Aguwa, 1983).

Animal, management and dietary treatments

A total of 225 six-week-old unsexed weaned New Zealand White rabbits were randomly distributed into 5 treatments until 14 weeks of age, each contained three

replicates (15 rabbits in each replicate). Dietary treatment included: a control diet (C), control diets plus 0.25 and 0.5% dried eucalyptus leaves (T1 and T2), and control diets plus 0.05 and 0.1% aqueous extract of dried eucalyptus leaves (T3 and T4). The basal diet (Table 1) was formulated according to NRC (1977). Rabbits were raised in cages which provided with a manual feeder and clean fresh water. Feed and water were provided *ad-libitum*. Rabbits were kept under the same hygienic and environmental conditions during the experimental period.

Growth performance

Live body weight (LBW) and feed intake (FI) were determined biweekly throughout the experimental period, and body weight gain (BWG) and feed conversion ratio (FCR) were calculated.

Nutrient digestibility coefficients

At the end week of the experimental period (14 weeks of age) four males in each treatment were used for determining nutrient digestibility coefficient of the tested diets. Rabbits were individually housed in metabolic cages; diets and fresh water were daily provided to the rabbit. Samples of feed and feces were collected for 3 days. Each animal feces was mixed and dried at 60 oC for 24 h. Chemical analyses of diets and feces were done according to the classical (AOAC, 1996). The nutritive values of the experimental diets were calculated according to Cheeke (1987).

Table 1. Composition and calculated chemical composition of the basal diet

Feed Ingredients (%)		Chemical composition (%DM basis)	
Alfalfa hay (12%)	27	Dry Matter%	89.71
Soybean meal (44%CP)	17.65	Crude Protein%	17.14
Barley	20	Crude Fiber%	12.75
Yellow corn	11.4	Ether Extract%	2.37
Wheat bran	17.5	Calcium %	1.19
Molasses	3	Total Phosphorus %	0.8
Limestone	0.75	Lysine %	0.89
Dicalcium - phosphate	1.9	Methionine %	0.5
Sodium chloride	0.3	Met + Cys %	0.79
DL-Methionine	0.2		
Mineral-vitamin premix*	0.3		
Total	100		

***Vit. And Min mixture: Each 3 kg contain: 6000000 IU Vit. A; 900000 IU Vit. D3; 40000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 10000 mg Pantothenic acid; 50000 Niacin; 3000 mg Folic acid; 250000 mg Choline; 8500 mg Mn; 50000 mg Zn; 50000 mg Fe; 200 mg I; 100 mg Se, 5000 mg Cu, and 100 mg Co.**

Haematologic and biochemical blood parameters

At the end of the growth experiment, fresh blood samples from three rabbits of each treatment were collected and delivered to the laboratory within 2 h of collection to determine blood picture constituents. Feldman *et al*. (2000) method was used to count RBC's and WBC's. The hemoglobin concentration (HGB) (g/dl) and packed cells volume percentages was estimating according to Drew *et al*. (2004)

For biochemical blood parameters other blood samples (3 samples of each treatment) were collected in clean heparinized tubes. Plasma was obtained by blood centrifugation at 3000 rpm for 20 min for analysis the blood biochemical parameters. Blood plasma total protein, albumin, triglycerides, total cholesterol, calcium, creatinine, uric acid, alanine amino transferase (ALT) and aspartate amino transferase (AST) were estimated by using commercial Kits. The globulin values were obtained by subtracting the values of albumin from the values of total proteins.

Statistical analysis

Data were analyzed using the GLM procedure of SAS software (SAS, 2001). Differences between treatments were assessed using Duncan's multiple range tests (1955) ($P<0.05$). The statistical model performed was as follow:

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

Where, Y_{ik} **= An observation,** μ **= Overall mean,** T_i **= Effect of treatments (** $i = 1,2\ldots.5$**),** e_{ik} **= random error**

RESULTS AND DISCUSSION

Growth performance

Effects of supplemented diets with Eucalyptus dried leaves (EGL) or its aqueous extract (EGE) on growth performance are reported in Table 2. Dietary supplementation of EGL or EGE had no effect on LBW during 6-10wk of age, but during 12-14wk, LBW increased compared to the control. Rabbits fed diet supplemented with EGL or EGE had greater BWG compared to the control, and the highest value was reported to T1 (supplemented with 0.25% EGL) increased by 21.25% compared with C, furthermore, there were no significant differences among T2, T3 and T4. Supplemented diets with EGL or EGE had no effect on feed intake by at the age 6-12wk. Feed intake tended to be decreased for rabbits fed on supplemented diets compared to control diet. Rabbits fed different levels of EGL or EGE (T1, T2, T3 and T4) recorded an improvement ($P<0.01$) of FCR as compared to group C. However, T1 and T3 groups are better than other groups (C, T2 and T4). Supplemented diets with EGL or EGE improved FCR compared with the control by 22.48, 16.67, 19 and 9.3% for T1, T2, T3 and T4, respectively. However, considering the entire experimental period, rabbit growth performance was improved by dietary treatments and growth parameters at slaughter were significantly different among groups, and the best results recorded to T2 which supplemented with 0.25% EGL.

Barbour *et al*. (2011) working with broilers and Hassan *et al*. (2011) with Japanese quail found that using eucalyptus as feed additives had a positive effect on growth performance, and this is may be due to the improvement in gut microflora and immunity system. Karimi *et al*. (2017) found that supplementing broiler diets with high level of Eucalyptus (3mg/kg) decreased body weight gain. The improvement in growth performance with EGL and EGA supplementation may be due to that eucalyptus has a positive effect of on primary antibody response (Barbour *et al*. 2008).

Nutrients digestibility coefficients

Results of digestibility coefficients for DM, OM, CP, CF, EE, NFE, and nutritive values (DCP, and TDN) of the experimental diets showed that there were no significant differences between treatments on DM, CF, EE and NFE digestibility coefficients. Coefficients of OM and CP digestibility were significantly improved ($P<0.05$) with supplement under study (Table 3). There were no difference among treatments (T_1, T_2, T_3 and T_4) in CP digestibility coefficients, whereas, coefficients of CP

digestibility were significant increased (P<0.05) in comparison with control. Addition of EGL and EGE in rabbit diets significantly (P<0.05) increased nutritive values in terms of TDN and DCP compared to control diet. The positive effect of EGL and EGE on the digestibility traits may be due to that eucalyptus improved immunity in broiler (Barbour *et al*., 2011). Also, eucalyptus has an antioxidante activity (Vratnica *et al*., 2011) which reduces the oxidative stress and improves heath condition (Sahin *et al*., 2010; Starčević *et al*., 2015).

Table 2. Growth performance of growing rabbits fed experimental diets.

Items	Treatments					Pooled SE	Sig
	Control	T_1	T_2	T_3	T_4		
			Live body weight (LBW), g				
6 week	703	705	698	702	698	1.01	NS
8 week	1108	1135	1200	1173	1115	32.48	NS
10 week	1547	1597	1598	1560	1588	8.01	NS
12 week	1908b	2000a	1987a	1963ab	1948ab	14.66	*
14 week	2240c	2568a	2416b	2433b	2390b	32.6	**
			Body weight gain (BWG), g				
6-8 week	405	430	501.5	471.6	416	25.6	NS
8-10 week	438.3^{ab}	461.7^{ab}	398.4^{ab}	386.67^{b}	473.3^{a}	18.13	*
10-12 week	361.6	403.5	388.33	403.3	360	34	NS
12-14 week	331.7^{c}	568.4^{a}	430^{b}	470^{b}	441.8^{b}	20.65	**
Total	1536.7^{c}	1863.3^{a}	1718.6^{b}	1731.8^{b}	1691^{b}	30.5	**
			Feed intake (FI), g				
6-8 week	523.61	528.06	558.06	540.28	536.11	5.44	NS
8-10 week	675.19	617.33	639.44	586.11	683.33	27.03	NS
10-12 week	1223.33	1138.4	1157.67	1088.3	1188.3	40.9	NS
12-14 week	1555	1375	1343.3	1411.7	1543.3	16.79	NS
Total	3977.1^{a}	3658.7^{bc}	3698.5^{abc}	3626.4^{c}	3951.1^{ab}	25.51	*
			Feed conversion ratio (FCR)				
6-8 week	1.3	1.24	1.12	1.15	1.29	0.01	NS
8-10 week	1.55	1.34	1.62	1.52	1.45	0.03	NS
10-12 week	3.44	2.94	3	2.73	3.31	0.24	NS
12-14 week	4.72a	2.42c	3.14bc	3.01bc	3.53b	0.15	**
Total	2.58a	2c	2.15bc	2.09c	2.34b	0.01	**

a, b and c: Means in the same row having different superscripts differ significantly .
C Control group, T^1 C + 0.25% EGL, T^2 C + 0.50% EGL, T^3 C + 0.05% EGE and T^4 C + 0.10% EGE

Table 3. Nutrient digestibility coefficients and nutritive values of the experimental diets.

Items	Treatments					Pooled SE	Sig
	Control	T_1	T_2	T_3	T_4		
DM	64.5	66.52	66.79	66.85	65.91	1.91	NS
OM	76.6^{b}	76.86^{ab}	77.96^{a}	77.91^{a}	77.73^{ab}	0.37	*
CP	72.91^{b}	73.79^{a}	74.42^{a}	73.84^{a}	74.62^{a}	0.33	*
CF	54.31	55.2	54.8	55.91	56.17	2.17	NS
EE	60.48	61.31	61.73	61	61.59	0.81	NS
NFE	81.16	81.51	82.63	82.72	82.57	0.61	NS
			Nutritive value (%DM)				
DCP	12.36^{b}	12.51^{a}	12.62^{a}	12.53^{a}	12.65^{a}	0.007	**
TDN	69.62^{c}	70.13^{bc}	70.85^{ab}	70.91^{b}	71.02^{a}	0.2	**

a, b and c: Means in the same row having different superscripts differ significantly.
C Control group, T^1 C + 0.25% EGL, T^2 C + 0.50% EGL, T^3 C + 0.05% EGE and T^4 C + 0.10% EGE

Hematological blood picture

Results presented in Table 4 show that, values of hemoglobin concentration and RBC's count were significantly (P<0.05) increased in growing rabbits fed diet supplemented with EGL or EGE compared to the control. Also, counts of WBC's and its fractions of lymphocytes were significantly (P<0.01) increased as both forms of eucalyptus were included in the diets. Other WBC's fractions, MCH and MCHC were not significantly affected by treatments. AbdEl-Motaal *et al*. (2008) found that lymphocytes count increased with using eucalyptus as feed additives in laying hen, lymphocytes count is an index of stress conditions (Gross and Siegel, 1985), since lymphocytes decrease when hens are stressed. Besides, the lymphocyte is considered a good indicator of the increase in immune efficiency (Wieslaw *et al*., 2006).

Metabolic blood parameters

Blood plasma parameters of the experimental treatments (total protein, albumin, globulin, triglycerides, total cholesterol, creatinine and the activities of AST and ALT enzymes for 12 week old NZW rabbits are shown in Table 5. The obtained results show that the experimental treatments did not have significant effects on globulin, creatinine and AST and ALT activities. Total protein and albumin were significantly (P<0.01) increased, while triglycerides (P<0.01) and total cholesterol (P<0.05) were significantly decreased with EGL and EGE feeding. AbdEl-Motaal *et al*. (2008) reported that using eucalyptus as feed additives in laying hen significantly increased plasma globulin and reduced the activities of AST and ALT. Ibrahim *et al*. (2018) reported that eucalyptus oil reduced cholesterol concentration in broiler chickens

Table 4. Hematological parameters of the studied treatments

Items	Treatments					Pooled SE	Sig
	Control	T_1	T_2	T_3	T_4		
Hemoglobin(g/dl)	8.77^{b}	9.93^{a}	10.73^{a}	9.87^{a}	10.1^{a}	0.31	*
Hematocrit value (HCT) %	47.27	47.05	48.43	47.59	48.74	1.01	NS
Red blood cells ($N\times10^{6}/cmm^{3}$)	3.7^{c}	4.07^{bc}	4.67^{ab}	4.14^{bc}	4.97^{a}	0.17	*
White blood cells ($N\times10^{3}/cmm^{3}$)	6.5^{b}	7.37^{a}	7.5^{a}	7.4^{a}	7.33^{a}	0.06	**
Neutrophil (N) (%)	35.47	35.37	35.57	32.87	34.27	8.33	NS
Lymphocytes (L)(%)	51.47^{b}	54.3^{ab}	56.7^{a}	55.2^{a}	57.97^{a}	3.64	**
Monocytes (%)	5.13	4.8	4.73	5.27	5.2	0.27	NS
Eosinophils (%)	3.53	4.07	4.5	4.47	4.5	0.27	NS
Basophils (%)	0.36	0.41	0.38	0.36	0.38	0.002	NS
Packed cell volume (PCV) (%)	28.06	28	28.63	30.2	30.17	0.29	NS
Mean cell volume (MCV) (fl)	85.3	83.6	84.9	83.3	82.2	0.41	NS
Mean corpuscular hemoglobin (MCH) (pg)	29.2	31	30.1	31.6	30.3	0.34	NS
Mean corpuscular hemoglobin concentration (MCHC) (g/dl)	24.2	26	24.6	24.6	24.3	0.09	NS

a, b and c: Means in the same row having different superscripts differ significantly.
C Control group, T^1 C + 0.25% EGL, T^2 C + 0.50% EGL, T^3 C + 0.05% EGE and T^4 C + 0.10% EGE

Table 5. Blood metabolic parameters of growing rabbits fed experimental diets.

Items	Treatments					Pooled SE	Sig
	Control	T_1	T_2	T_3	T_4		
Total protein (g/dl)	5.47^{b}	5.83^{ab}	5.9^{a}	5.73^{ab}	6^{a}	0.04	*
Albumin (g/dl)	3.8^{b}	3.97^{ab}	3.9^{ab}	4.03^{bc}	4.16^{a}	0.02	*
Globulin (g/dl)	1.67	1.87	2.03	1.7	1.83	0.04	NS
Triglycerides (mg/dl)	34.67^{a}	34^{a}	32.67^{ab}	33^{ab}	30.67^{b}	1.8	*
Total cholesterol (mg/dl)	54.67^{a}	55.67^{a}	51^{ab}	49^{b}	46^{b}	7.33	**
Creatinine (mg/dl)	1.08	1.87	1.08	1.1	1.05	0.01	NS
AST (IU/ml)	37	38	36.67	36	39.67	12.13	NS
ALT (IU/ml)	26	24.33	26	26.33	23.1	7.33	NS

***a*, b and c: Means in the same row having different superscripts differ significantly.**
C Control group, T^1 C + 0.25% EGL, T^2 C + 0.50% EGL, T^3 C + 0.05% EGE and T^4 C + 0.10% EGE

CONCLUSION

It could be concluded that eucalyptus globules leaves or its aqueous extract is a good feed additive in growing New Zeland growing rabbit diets as it improve growth performance, nutrients digestibility coefficients and had no adverse effects on blood constituents.

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الأداء الإنتاجي ومعاملات الهضم وبعض قياسات الدم في الأرانب النيوزلاندي النامية المغذاة علي الكافور كاضافات غذائية

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أجريت هذه الدراسة لمعرفة تأثير إضافة مستويات مختلفة من الأوراق المجففة أو المستخلص المائي لأوراق الكافور علي الأداء الإنتاجي ومعامل الهضم وبعض صفات الدم ، تم استخدام 225 أرنب نيوزلاندي النامية المفطومة في هذه الدراسة ، وكانت مجموعة المعاملات: مجموعة مقارنة (الكنترول) والمجموعة الثانية والثالثة مضاف اليها ورق الكافور المجفف بنسبة 0.25 و 0.5 ٪ والمجموعة الرابعة والخامسة مضاف اليها مستخلص الكافور المائي بنسبة 0.05 و 0.1 ٪ ، واستمرت تجربة النمو لمدة ثمانية أسابيع ، وتم عمل تجربة هضم في نهاية التجربة ، ثم ذبحت الأرانب أخذت عينات الدم من الحيوانات ، وتم اخذ قياسات الدم. ويمكن تلخيص النتائج كالتالي: امداد عليقة الارانب بورق الكافور المجفف أو المستلخص المائي للكافور ادي لزيادة وزن الجسم الحي ومعدل الزيادة في الوزن ومعامل التحويل الغذائي خاصة للمعاملة التي تحتوي علي 0.25٪ أوراق كافور مجففة مقارنة مع المجموعة المقارنة، وكانت أفضل نتيجة لمعامل التحويل الغذائي للمجموعة المحتوية علي 0.25٪ ورق كافور مجفف تليها المجموعة المحتوية علي 0.05٪ المستخص المائي لأوراق الكافور ، أدت المعاملات إلي زيادة معاملات الهضم لكل من المادة العضوية والبروتين الخام والقيم الغذائية للمركبات الغذائية الكلية المهضومة و البروتينات الخام المهضومة، إضافة أوراق الكافور المجففة أو المستخلص المائي لأوراق الكافور أدت إلي زيادة الهيموجلوبين وعدد كرات الدم الحمراء وكرات الدم البيضاء والنسبة المئوية للخلايا الليمفاوية ، أدي إضافة أوراق الكافور المجففة أو المستخلص المائي لأوراق الكافور إلي زيادة معنوية في البروتين الكلي والألبيومين ونقص الكوليستيرول الكلي و الجلسيريدات الثلاثية وليس له تأثير علي الكرياتينين وانزيمات الكبد ، يمكن أن نستخلص أنه يمكن أستخدام الكافور كاضافات غذائية ادي لتحسن الأداء الإنتاجي ومعامل الهضم وليس له تأثير عكسي علي قياسات الدم.