



# Effect of Injection with Clove Oil or Salbutamol Drug (A Betaadren-ergic Agonist) as a Growth Promoter on Productive and Some Physio-logical Responses of Growing Rabbits

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**Abstract** | This study aimed to compare the effectiveness, safety and adverse effects of clove oil with those of salbutamol drug on growth performance, carcass characteristics and some blood parameters for growing rabbits. Forty New Zealand White (NZW) rabbits aged 6 weeks and weighing 950±30 g were equally and randomly distributed into five groups which containing eight rabbits in each. Rabbits were injected once-weekly for all experimental period up to 14<sup>th</sup> weeks of age. The first treatment groups were the control without injection (T1), and those in 2<sup>nd</sup> and 3<sup>rd</sup> groups were injected with clove oil (25.0 “T2” and 12.5 “T3” µml/ rabbit/week), respectively. whereas, 4<sup>th</sup> and 5<sup>th</sup> groups were injected with Salbutamol (50 “T4” and 25 “T5” µml/rabbit/week). The significant (P<0.05) increased in growth performance was observed in rabbits injected with low level of clove oil (T3) compared with other groups. Furthermore, highly significant effect on relative weight of fur and testosterone hormone levels were observed in rabbits injected with low level of clove oil (T3) compared with other treatment groups. In conclusion, clove oil at 12.5 µml., may be useful for improving growth performance, and increases the release of Gonadotropin-releasing hormone.

**Keywords** | Clove oil, Growth performance, Hormones, Rabbits, Salbutamol

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## INTRODUCTION

Rabbits are played an important role in solving production meat deficiency; this is due to the rapid rate of growth and rabbits reproduction, developing early-maturing, enhance the efficiency of feed utilization and high genetic selection potential particularly in the developing countries (Khalil, 2002).

Previous studies showed that salbutamol solution belongs to a class of medications called bronchodilators especially with mild or moderate airways obstruction, and more specifically,  $\beta_2$ -adrenergic agonists and make breathing easier (Hughes et al., 1987; Tantucci et al., 1998; Aliverti et al., 2005).

Salbutamol as  $\beta$ -adrenergic agonists ( $\beta$ -agonists) are functionally comparable to Catecholamines which could be stimulated carbohydrate metabolism causing quick changes in serum carbohydrate level, exert effects on carcass yield and improve growth performance (Smith, 1998). This is due to increase protein synthesis in myofibrillar and skeletal muscle (Fawcett et al., 2004) and adipocytes metabolism and decrease carcass fat (Mersmann, 2002; Avendaño-Reyes et al., 2006). Natural substances are commonly used in the production of medicinal, as well as in agriculture. In recent years, there has been an increased interest in biologically active plant extracts (El-Kholy et al., 2018, 2021), especially, essential oils which consists of complex mixtures of aromatic compounds extracted by distillation

from various part of plants (Bakkali et al., 2008). The literature provides information on numerous characteristics of essential oils, such as antibacterial, antifungal, antiviral, antioxidant, or immunostimulatory properties (Hood et al., 2010; Bharti et al., 2012; Solorzano-Santos and Miranda-Novales, 2012; Alali et al., 2013; Bento et al., 2013; Mahboubi et al., 2013; Krishan and Narang, 2014) and antidiarrheal agents (Gopi et al., 2014). The compounds found in the essential oil of cloves are eugenol, eugenol acetate, and B-caryophyllene. The structures of eugenol, eugenol acetate, and caryophyllene. Eugenol is a principal compound responsible for giving cloves their taste and distinctive aroma (Bisergaeva et al., 2021). Clove, and its essential oil, is one of the plant extracts that has been found effective in poultry to improve growth performance, control some intestinal pathogens, acts antiseptic and as digestion stimulant, and shows strong antimicrobial and antifungal, anti-inflammatory, anesthetic, anti-carcinogenic, antiparasitic and antioxidant effects (Najafi and Torki, 2010; Chami et al., 2005; Mitsch et al., 2004; Kamel, 2001). antiviral, antitumor and cytotoxic properties (Gayoso et al., 2005; Politeo et al., 2010). Nevertheless, it was observed that liver damage caused by a high doses of clove oil (Susan et al., 2010). The antimicrobial activity of clove oil is related to highly content of eugenol which influences on virulence factors is provided (Marchese et al., 2017).

There is little information on comparing the effects of these two agents until now. Accordingly, this study aimed to compare the effectiveness, safety and adverse effects of clove oil to those of a beta-adrenergic agonist drug (salbutamol) on growth performance, carcass characteristics and some blood metabolites in rabbits.

## MATERIALS AND METHODS

The experimental work of the present study was carried out at the experimental animals' center belonging to Station of EL- Serw Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, from September 2019 to November 2019.

### ETHICAL APPROVAL

This research was carried out in accordance with the Animal Care and Use Committee guidelines of the Damietta University, Damietta, Egypt (Approval number: 03/2018/du.edu). The rabbits in the experiment were provided proper care and management without unnecessary discomfort.

### RABBITS AND MANAGEMENT

A total number of 40 unsexed, six weeks old, New Zealand White (NZW) were divided and assigned randomly into five experimental groups of 8 rabbits each with an average live body weight of 950±30 g. The five experimental groups

were as follows: The control group (T1; no injection), while groups T2 and T3 were subcutaneous injected once-weekly with clove oil (25 and 12.5 µml/ rabbit), respectively. The other experimental groups (T4 and T5) were also subcutaneous injected once-weekly with Salbutamol (50 and 25 µml/ rabbit); respectively. Rabbits were fed a commercial growing diet formulated to cover the recommended requirements of post-weaning rabbits according to the National Research Council (NRC, 1977) from 6 to 14 weeks of age as shown in Table 1. All the experimental animals were healthy and clinically free from external and internal parasites and were kept in the same management and hygienic conditions.

**Table 1:** Composition and calculated analysis of a commercial diet.

Ingredients	%
Yellow corn	9
Barley	15
Wheat bran	30
Soybean meal (44%)	8
Alfalfa hay	35
Di-calcium phosphate	1.2
Limestone	1.0
Salt	0.5
Mineral-vitamin premix <sup>(1)</sup>	0.3
Total	100
<b>Calculated chemical analysis<sup>2</sup></b>	
Digestible energy (kcal/kg)	2732
Crude protein %	16.52
Crude fiber %	13.18
Ether extract %	2.96
Calcium%	1.23
Phosphorus%	0.81
Available phosphorus%	0.53
Lysine%	0.78
Methionine%	0.33
Price/kg(L.E)	4.58

<sup>1</sup>One kilogram of mineral-vitamin premix provided: Vitamin A, 150,000 UI; Vitamin E, 100 mg; Vitamin K3, 21mg; Vitamin B1, 10 mg; VitaminB2, 40mg; Vitamin B6, 15mg; Pantothenic acid, 100 mg; Vitamin B12, 0.1mg; Niacin, 200 mg; Folic acid, 10mg; Biotin, 0.5mg; Choline chloride, 5000 mg; Fe, 0.3mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1mg; and Zn, 450mg. <sup>2</sup>Calculated according to NRC (1977).

### MEASUREMENTS

Growth performance of NZW rabbits during the whole experimental period (6-14 weeks) was assessed by live body weight (LBW), body weight gain (BWG), feed consumption (Fc) and feed conversion ratio (FCR). At

the end of experimental period (14 weeks of age), five rabbits from each group were randomly chosen and slaughtered after complete bleeding, three blood samples were individually collected from each rabbit each group. These blood samples were centrifuged at 3500 rpm for 20 min. then stored at  $-20^{\circ}\text{C}$  until analysis. Rabbits were individually weighed, and immediately their carcasses with fur and legs were skinned and then eviscerated. Giblets (including heart, liver and kidney) and abdominal fat contents were weighed, all carcass traits were expressed as % of live body weight.

### BLOOD SAMPLES

Serum samples from slaughtered rabbits (3/treatment) were analyzed, using commercial kits (Immunotech Corp France) for the determination of level of aspartate (AST, U/L) and alanine amino transaminases (ALT, U/L) using commercial kits (Linear Chemicals, Barcelona, Spain) according to the manufacturer procedure. Also, the serum was assayed for total cholesterol (TC, mg/dl), total glycerides (TG, mg/dl), high-density Lipoprotein (HDL, mg/dl), and Low-Density Lipoprotein (LDL, mg/dl) using standard protocol methods (Vogel and Vogel, 1997). The serum levels of Immunoglobulin G (IgG) was determined by ELISA kits (Kamiya Biomedical Company, USA) following the instructions enclosed in the manufactured kits (Elabscience Company, Wuhan, China). Serum concentrations of follicle-stimulating hormone (FSH, ng/ml) and luteinizing hormone (LH, ng/ml) were measured by using ELISA kits (Elabscience Biotechnology Co., Ltd, www.elabscience.com). Serum thyroxine (T4, ng/ml) were determined in sera using the ELISA technique according to Walker (1977). According to Koracevic et al.

(2001), serum samples were also used to determine total antioxidant capacity (TAC, ng/ml). The concentrations of testosterone in the serum blood were determined using commercial immunoassay kits (Monobind, Inc., Lake Forest, CA 92630, USA).

### STATISTICAL ANALYSIS

Statistical analysis for the obtained data was performed by analysis of variance using a one-way analysis of variance (SAS, 2004) using the following fixed model:

$$Y_i = \mu + T_i + e_i$$

Where;  $Y_i$  = The observation;  $\mu$  = Overall mean;  $T_i$  = Effect of treatments ( $i = 1, 2, 3$  and  $4$ );  $e_i$  = Random error component assumed to be normally distributed. Duncan's multiple range test was used to separate significant differences among means (Duncan, 1955).

## RESULTS AND DISCUSSION

### GROWTH PERFORMANCE

No significant differences among experimental groups regarding to the growth performance parameters (initial body weight, final body weight and body weight gain at different experimental periods (10 or 14 wks of age). Nevertheless, clove oil levels had a significant effect on total body weight gain, feed intake and feed conversion ratio during whole experimental period (6-14 wks of age), as shown in Table 2. These results may be due to the active component in clove oil (eugenol) which was documented as improvement digestion, increase the performance of poultry and has the property antimicrobial activity against intestinal bacteria, additionally, activation bile salt secretion

**Table 2:** Effect of salbutamol or clove oil on growth performance of growing rabbits.

Measurements	Control	Clave oil administration ( $\mu\text{ml}/\text{rabbit}$ )		Salbutamol administration ( $\mu\text{ml}/\text{rabbit}$ )		SEM	P Value
	T1 (0)	T2 (25)	T3 (12.5)	T4 (50)	T5 (25)		
BW (6 Wks)	936.60	999.43	1030.66	994.70	992.25	63.14	0.883
BW (10 Wks)	1634.06 <sup>b</sup>	1801.69 <sup>ab</sup>	1893.94 <sup>a</sup>	1726.31 <sup>ab</sup>	1805.56 <sup>ab</sup>	70.34	0.129
BW (14 Wks)	2391.4	2602.0	2627.6	2504.9	2633.5	85.79	0.236
BWG (6-10 Wks)	697.46 <sup>c</sup>	802.26 <sup>ab</sup>	863.28 <sup>a</sup>	731.61 <sup>bc</sup>	813.31 <sup>ab</sup>	29.57	0.003
BWG (10-14 Wks)	757.31	800.34	733.69	778.59	827.97	38.47	0.470
BWG (6-14 Wks)	1454.78 <sup>b</sup>	1602.61 <sup>ab</sup>	1596.96 <sup>ab</sup>	1510.21 <sup>ab</sup>	1641.28 <sup>a</sup>	52.37	0.098
FI (6-10 Wks)	2800 <sup>c</sup>	2914 <sup>ab</sup>	2579 <sup>c</sup>	2855 <sup>bc</sup>	3004 <sup>a</sup>	37.71	<0.0001
FI (10-14 Wks)	3103 <sup>ab</sup>	3089 <sup>ab</sup>	2717 <sup>c</sup>	2967 <sup>b</sup>	3166 <sup>a</sup>	58.15	<0.0001
FI (6-14 Wks)	5903 <sup>b</sup>	6003 <sup>ab</sup>	5296 <sup>c</sup>	5822 <sup>b</sup>	6170 <sup>a</sup>	83.29	<0.0001
FCR (6-10 Wks)	4.07 <sup>a</sup>	3.66 <sup>a</sup>	3.02 <sup>b</sup>	3.95 <sup>a</sup>	3.75 <sup>a</sup>	0.18	0.002
FCR (10-14 Wks)	4.16	3.92	3.78	3.86	3.90	0.20	0.744
FCR (6-14 Wks)	4.10 <sup>a</sup>	3.78 <sup>a</sup>	3.33 <sup>b</sup>	3.89 <sup>a</sup>	3.80 <sup>a</sup>	0.15	0.018

T1: control; T2: was injected with 25  $\mu\text{ml}$  clove; T3: was injected with 12.5  $\mu\text{ml}$  clove; T4: was injected with 25  $\mu\text{ml}$  salbutamol; T5: was injected with 50  $\mu\text{ml}$  salbutamol. Initial body weight (BW6Wks.); body weight gain (BWG); Feed intake (FI); Feed conversion ratio (FCR); Standard error of the mean (SEM). <sup>a,b,c</sup> Means in the same raw with different superscripts differ significantly ( $P \leq 0.05$ ).

and digestive enzyme activities in the intestinal mucosa and pancreas (Hernandez et al., 2004; Jang et al., 2007; Mehr et al., 2014). In contrary, negative effect on FCR were recorded on either high or low levels of salbutamol group in compare to other treated groups. On the other hand, BWG at whole experimental period (6-14 wks) was significantly ( $P<0.05$ ) higher in salbutamol groups (T4 and T5) in compared to control group (T1). The trends resulting from salbutamol injection in the present study are in agreement with study involving quails received 7 mg Salbutamol/kg diet (Zare-Shahneh et al., 2012).

An increased level of clove oil led to a negative effect on performance. The current results agree with those of Mohammadi et al. (2014) and Al-Mufarrej et al. (2019) who found that high level of clove oil had decreased growth performance and total feed intake. However, Mahrous et al. (2017) observed that no significant differences in growth performance (BWG and FCR) when broiler chickens fed low level of clove, but performance deteriorated in groups supplemented with higher levels of cloves.

An improvement in weight gains in groups injection with Salbutamol-treated in the present study could be associated with the fact that betaadrenergic compounds decrease degradation of protein (Forsberg and Merrill, 1986) and increase protein deposition (Etherton and Smith, 1991).

### CARCASS CHARACTERISTICS

Table 3 revealed that treatments had no significant effect on all carcass characteristics at 14 weeks of age except weights of fur. However, relative weights of fur were significantly lower ( $P<0.05$ ) in rabbits treated with salbutamol in

compared to other experimental groups. In spite of neither clove oil nor salbutamol had statistical differences in percentages of carcass yield but treated groups showed numerically increased in compared to control one (T1). This result is consistent with some reports (El-Nomeary et al., 2020) in rabbits using other essential oils and (Zare Shahneh et al., 2012) in quail. It seems that the literature is still sparse on the effect of clove oil and salbutamol on rabbit's carcass traits.

The increasing carcass yield % for treated groups may be mainly related to the increase in growth performance in these groups. As a result, pre-slaughter weight is regarded as one of the most essential factors influencing rabbit carcass yield percentage.

### BLOOD SERUM PARAMETERS

The results in Table 4 showed that low dose of salbutamol (T5) leads to increase of liver function and increased serum lipid profiles except LDL. This might be due to toxic effects of salbutamol. Despite, liver enzymes and serum lipid profiles were not significantly different in each clove oil levels and control group. These results agreement with findings of Abdel-Azeem et al. (2010); El-Kholy et al. (2018, 2020) who showed that plasma ALT and AST concentrations were not significantly affected by adding natural phytobiotics for growing rabbit and broiler diets. Additionally, low level of clove oil had positive effect on immunoglobulin G (IgG), Testosterone, LH and thyroxin hormones compared to other groups. On the other side, clove oil levels or salbutamol levels revealed insignificant ( $P<0.05$ ) changes in serum levels of TAC, FSH and HDL in all group (Table 4).

**Table 3:** Effect of salbutamol or clove oil on growth performance of growing rabbits.

Measurements	Control	Clave oil administration (µml/rabbit)		Salbutamol administration (µml/rabbit)		SEM	P value
	T1(0)	T2 (25)	T3 (12.5)	T4 (50)	T5(25)		
Body weight	2391.4	2602.0	2627.6	2504.9	2633.5	85.79	0.236
Weight after slaughter %	97.06	97.02	98.26	97.60	96.32	1.02	0.732
Carcass yield %	60.76	61.49	62.26	63.73	63.24	1.32	0.524
Weight of fur %	17.52 <sup>ab</sup>	16.45 <sup>bc</sup>	18.13 <sup>a</sup>	16.08 <sup>c</sup>	16.10 <sup>c</sup>	0.35	0.007
Digestive tract weight %	14.24	14.71	14.46	13.14	13.37	1.11	0.814
Liver weight %	2.81	3.03	2.76	2.82	2.54	0.22	0.662
Heart weight %	0.21	0.24	0.25	0.26	0.22	0.02	0.507
Testicular weight %	0.40	0.37	0.34	0.37	0.39	0.05	0.915
Kidney weight %	0.71 <sup>a</sup>	0.53 <sup>b</sup>	0.63 <sup>ab</sup>	0.70 <sup>a</sup>	0.58 <sup>ab</sup>	0.05	0.107
Lungs weight %	0.68	0.60	0.49	0.62	0.50	0.06	0.146
Spleen weight %	0.05	0.04	0.04	0.06	0.05	0.01	0.544

T1: control; T2: was injected with 25 µml clove; T3: was injected with 12.5 µml clove; T4: was injected with 25 µml salbutamol; T5: was injected with 50 µml salbutamol. Standard error of the mean (SEM). <sup>a, b, c</sup> Means in the same raw with different superscripts differ significantly ( $P\leq 0.05$ ).



**Table 4:** Effect of salbutamol or clove oil on lipid profile, liver function and immunoglobulin of growing rabbits.

Measurements 1	Control	Clave oil administration (µml/rabbit)			Salbutamol administration (µml/rabbit)		SEM	P value
	T1 (0)	T2 (25)	T3 (12.5)	T4 (50)	T5 (25)			
AST U/ml	53.7 <sup>ab</sup>	50.3 <sup>b</sup>	51.0 <sup>ab</sup>	56.0 <sup>ab</sup>	61.3 <sup>a</sup>	3.15	0.171	
ALT U/ml	3.33	3.00	2.67	1.67	2.33	0.54	0.296	
Chol. mg/dL	63.50 <sup>a</sup>	57.67 <sup>a</sup>	61.67 <sup>a</sup>	37.33 <sup>b</sup>	73.33 <sup>a</sup>	4.91	0.005	
TG mg/dL	43.67 <sup>ab</sup>	38.00 <sup>ab</sup>	35.33 <sup>b</sup>	49.67 <sup>ab</sup>	77.67 <sup>a</sup>	12.13	0.176	
HDL mg/dL	30.17	20.67	23.33	21.00	34.00	4.13	0.158	
LDL mg/dL	28.27 <sup>a</sup>	22.00 <sup>a</sup>	27.73 <sup>a</sup>	6.40 <sup>b</sup>	23.80 <sup>a</sup>	3.22	0.004	
FSH ng/ml	0.10	0.11	0.11	0.10	0.10	0.01	0.630	
LH ng/ml	0.18 <sup>ab</sup>	0.20 <sup>a</sup>	0.19 <sup>a</sup>	0.20 <sup>a</sup>	0.16 <sup>b</sup>	0.01	0.015	
Testosterone ng/ml	0.22 <sup>d</sup>	1.41 <sup>b</sup>	2.28 <sup>a</sup>	0.86 <sup>c</sup>	0.19 <sup>d</sup>	0.03	<.0001	
IgG mg/dL	339 <sup>d</sup>	710 <sup>b</sup>	743 <sup>a</sup>	720 <sup>b</sup>	401 <sup>c</sup>	3.87	<.0001	
T4 ng/ml	2.30 <sup>b</sup>	2.92 <sup>a</sup>	2.89 <sup>a</sup>	2.62 <sup>ab</sup>	2.47 <sup>b</sup>	0.10	0.007	
TAC (mM/L)	0.28	0.53	0.42	0.37	0.21	0.09	0.222	

T1: control; T2: was injected with 25 µml clove; T3: was injected with 12.5 µml clove; T4: was injected with 25 µml salbutamol; T5: was injected with 50 µml salbutamol. Standard error of the mean (SEM). <sup>a,b,c,d</sup> Means in the same raw with different superscripts differ significantly (P≤ 0.05). <sup>1</sup>AST: aspartate amino-transferase; ALT, alanine amino-transferase; Chol: total cholesterol; TG, total glycerides; HDL, High density lipoprotein; LDL, low density lipoprotein; FSH, follicle stimulating hormone; LH, Luteinizing hormone; IgG, immunoglobulin G; T4, Thyroxine; TAC, total antioxidant capacity.

The increase in FI and BWG in treated groups with low level of clove oil may explain our results of the significant increase in serum level of thyroxine hormone and IgG as compared to control group (Mukhtar, 2011).

Selva and Johnston (2004) and Calka (2006) found that increase in LH, FSH may be due to the release of norepinephrine by explant. Norepinephrine increases the synthesis of nitric oxide, which increases the release of Gonadotropin-releasing hormone (GnRH) from the hypothalamus as well as FSH and LH gonadotropins from the anterior pituitary gland.

## CONCLUSIONS AND RECOMMENDATIONS

From the obtained results, we concluded that injection with low level of cloves oils (12.5 µml/ rabbit) could significantly promote final body weight and weight of fur, additionally, improving feed conversion ratio and increases the release of Gonadotropin releasing hormone compared to other treatments. Hence, injection rabbit with clove oil could be a safe alternative to drugs, improvement growth production and produce harmless meat with numerous beneficial effects on consumer health.

## NOVELTY STATEMENT

We found that subcutaneous injections with either low level of cloves oils (0.125 µml/rabbit) or high level of salbuta-

mol (50 µml/rabbit) could significantly promote final body weight and weight of fur, additionally, improving feed conversion ratio and increases the release of gonadotropin-releasing hormone during fattening period of rabbits.

## AUTHOR'S CONTRIBUTION

KHE, YSR, ME and EAE developed the concept of the manuscript. All authors checked and confirmed the final revised manuscript.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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