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Research Article

Comparative effects of sesame oil and clove oil on growth performance, blood profile and immunity of rabbit (*Oryctolagus cuniculus*)

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Citation

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Abstract

Essential oils are being used to improve health due to their high nutritive value. In this study, the comparative effects of Sesame oil and Clove oil were assessed on growth performance, blood profile and immunity of male rabbits. A total of 108 male rabbits were divided into 3 treatment groups; Treatment group A (Sesame oil), Treatment group B (Clove oil), Treatment group C (Sesame oil + Clove oil). Each treatment group was further divided into 4 subgroups according to dosage design of oils. The control group was untreated, while treatment dose for groups A and B was divided into 3 different concentrations; 0.1, 0.2 and 0.3ml/kilogram Body Weight. Whereas, the treatment concentrations for group C were as; 0.1+0.1, 0.2+0.2 and 0.3+0.3ml/kilogram Body Weight of oils for 1-1.5kg of rabbits. The rabbits were managed in proper caging under controlled conditions of photoperiod (12 light: 12 dark cycles) 23±2ºC of temperature. The selected dose concentration of each oil was administered orally and once a day for six weeks. The results showed that both oils had significantly enhanced the growth performance and reduced the Feed Conversion Ratio. The hematological parameters were increased in all treatment groups, respectively. The Enzyme-linked Immunosorbent Assay for Rabbit Hemorrhagic Disease Virus also showed a significant increase in antibody titer level. The study showed that Sesame oil and Clove oil as dietary supplements have significantly ($p<0.05$) improved overall performance of male rabbits. The current study clearly indicated that both oils could be a better choices for health purposes.

Keywords: Essential oils; ELISA; Hematology; Immune system

Introduction

Essential oils are aromatic liquids, with their biological activities like antibacterial, antifungal, anti-oxidant, and anti-inflammatory properties [1]. The oils are basically known to be as antibiotic growth agents, used in animal diet to induce positive

impacts on growth performance, internal microbiota and health [2]. The bioactivity of the oils depends on the complex mixture of volatile and non-volatile compounds produced by the metabolism activity of aromatic natured plants [3].

Sesamum indicum is the oldest plant, belongs to the family Pedaliacecae and is mainly cultivated in tropical areas [4]. It is a stable edible oil with a high degree of unsaturation property, normally available as high-quality oil and rich in total phenolic content, flavonoids [5], lignans, tocopherol homologues, and phytosterols [6]. Oil is considered as a rich source of food because of calcium and phosphorus [7]. The seed contains 50-60% part of the oil, 18-25% of protein content, 13.5% of carbohydrates, and 5% of ash [8]. The oil is used to cure diseases and is helpful in lowering the concentration of cholesterol, controlling blood pressure, and dermatological disease management [9]. It also performs antioxidative, anti-hypersensitive, anti-cancer and anti-immunoregulatory activities [10]. It can cause allergic reactions but it is rare about 0.1% in one piece population [11]. It is used as solvent, skin softeners, margarines, pharmaceuticals and lubricants [12].

The Clove (*Syzygium aromaticum*) an aromatic flower bud of the tree that belongs to the family Myrtaceae, has the eugenol compound with beta-caryophyllene and has a lesser amount of the other constituents like benzyl alcohol in its oil [13]. The amount of eugenol is 78% with 13% beta-caryophyllene in the oil [14]. The chemical analysis shows that this oil has 36 components but 9 are with the highest concentrations [15]. It has antibacterial activity [16], a source of the new antifungal metabolites due to its phenolic constituents, the carvacrol, and the eugenol [17]. Clove oil has antioxidant properties and photocytotoxicity due to the eugenol [18, 19]. It also has antimutagenic potential, so it shows antitumor action [20]. Clove oil is a popular medication for dentistry [21], headache, sore throat, respiratory system and digestive system aliment [22]. At very low concentrations, clove oil acts as anti-oxidant and anti-inflammatory property, but at higher concentrations, it can cause tissue damage [23]. It can enhance the learning and memory recall processes [24]. Clove oil has phytochemical extracts [25], when this oil is given intravenously at low concentration, it mainly helps to reduce the fever in rabbits [26]. Although, both oils perform various functions, however, this study was designed to estimate the comparative effects of Sesame oil and Clove oil on the growth performance, blood profile and immunity of rabbits.

Materials and Methods

Experimental animal

Eight-week-old male rabbits (*Oryctolagus cuniculus*) with the weight of 1.0 to 1.5 kg were purchased from Ghazi Road Lahore Institute of Veterinary Research Lahore, Punjab, Pakistan during the month of February. These rabbits were vaccinated and acclimatized before experimentation for 15 days with optimum light i.e. 12 hour light and dark cycle and 23 ± 2ºC temperature [27]. Rabbits were kept in cages and were offered ad libitum feed and water, after every eight hours over the whole experiment. The selected concentration of essential oils was mixed in the soaked bread, given orally and once a day. To prevent from the disease and stress, the wasted food and feces of the rabbits were removed daily. Furthermore, bedding was also changed on daily basis to feel them comfortable, safe and warm.

Essential oils and dosage design

Oils of Sesame seeds and Clove buds were purchased from the local market. Rabbits were distributed into 3 treatment groups; Treatment group A (Sesame oil), Treatment group B (Clove oil) and Treatment Group C (Sesame oil + Clove oil). Each treatment group was further divided into 4 subgroups according to the dosage design of oils. The

control group was untreated, while treatment dose for groups A and B was divided into 3 different concentrations; 0.1, 0.2 and 0.3ml/kg BW. Whereas, the treatment concentrations for group C were as; 0.1+0.1, 0.2+0.2 and 0.3+0.3ml/kg BW. The selected dose of each oil was given once a day for six weeks. To reduce error, three replications per treatment were implemented [28, 29].

Growth performance (FCR)

After every 15th day of the experiment, the digital balance was used to check the weight of the rabbit in kilograms. The weight of each rabbit was checked after 12 hours of fasting, to avoid the addition of gut content in total body weight. The feed intake was fixed and measured for each group on daily basis. The feed consumption rate was also checked. The formula for feed conversion ratio was applied to get results [30].

FCR = Total Feed Intake / Total Weight Gain

Blood sampling

At 15th, 30th and 45th day of the research work, blood samples were collected through the jugular vein. About 2ml of blood sample was added into 3ml K_3 EDTA tubes for calculation of blood components. The same amount of blood was also added into the gel and clot activator tubes to extract the serum. After collecting the blood samples the specimens were treated with silver nitrate solution to halt the blood loss [31, 32].

Hematological tests and ELISA

The blood analysis was conducted in the Zoology Laboratory to check the levels of CBC count, via a Semi-Automatic Chemical Analyzer, model HKTE0112 Guangzhoun Hekang. Results were displayed numerically as well as graphically on the screen of the analyzer. Later on, these parameters were checked to identify the changes as compared to the control group [31, 33].

The serum was extracted by using centrifugation machine. Samples were centrifuged at the speed of 1600xg for 15 minutes at 4^{o}C to obtain the serum from the blood and separated in microtubes for test analysis [34]. To check the humoral responses, an indirect ELISA-Array assay was performed in the Zoology Laboratory. Then serum samples were used to run the ELISA test. The antibodies present in the serum sample were attached to colorless substrate and color product was obtained at the end of the test. So, the antigen and antibody combination was helpful to access the results during the ELISA procedure [35].

Data analysis

All the mean standard values were obtained with the help of the SPSS (Statistical Packages for Social Science). Standard Error Mean (±S.E.M) for each of parameters. Moreover, Variation between groups was detected by the ANOVA test along with post hoc test (Tukey HSD). The extent of variation found in the groups. The $P<0.05$ value was deliberated as statistically significant.

Results

Body weight and FCR

When Sesame oil was orally administered to rabbits, it was observed that body weight was significantly ($p<0.05$) increased (Fig. 1) at concentration 0.3ml/kg BW with mean value of 4.84 ± 0.02 kg (Table 1) and the FCR was significantly decreased (Fig. 2) with this mean value 4.05±0.04.

When Clove oil was orally administered to rabbits, it was examined that body weight increased (Fig. 3) at 0.2ml/kg BW concentration with mean value of 4.35 ± 0.00 kg (Table 2) and the FCR was significantly decreased (Fig. 4) with the mean value 4.05±0.04.

When the combination of both oils SO + CO was given to rabbits, it was observed that it has exhibited maximum weight gain (Fig. 5) at concentrations 0.3+0.3ml/kg BW with a mean value of 5.45 ± 0.01 kg (Table 3) and with decrease in FCR (Fig. 6) with a mean value of 3.80 ± 0.05 for all treated groups as compared to untreated groups.

Blood profile

The level of blood profile components was significantly increased (Table 4) throughout the study. Sesame oil has increased the blood components at concentration 0.3ml/kg BW. The mean values of Hb level (14.25 ± 0.38 g/dl), RBC's count (8.06 ± 0.12 10^12/l), HCT level (53.66 ± 0.88 %), MCV (83.66 ± 0.88 fL), MCH level (41.00 ± 1.52 pg), MCHC level (46.33 ± 0.88 g/dl), WBC's count (11.86 ± 0.13 10^9/l) and platelets number (342.33 ± 0.88 10^9/l) have showed the increase than the control group.

Clove oil has improved the blood profile at concentration 0.2ml/kg BW. The average values for Hb level (14.06 ± 0.46 g/dl), RBC's count (7.83 ± 0.03 10^12/l), HCT level (53.00 ± 2.30 %), MCV (83.66 ± 0.88 fL), MCH level (36.66 ± 0.33 pg), MCHC level (41.33 ± 1.20 g/dl), WBC's count (13.43 ± 0.23 10^9/l) and platelets number (392.00 ± 3.5110^9/l) have exhibit the increase as compared to the control group.

The hematological parameters of SO + CO treated groups were higher at concentrations 0.3+0.3ml/kg BW than other treatment and control group with mean values such as Hb level (16.03 ± 0.14 g/dl), RBC's count (9.76 ± 0.14 10^12/l), HCT level (58.66 ± 1.76 %), MCV (95.33 ± 1.76 fL), MCH level (47.66 ± 0.88 pg), MCHC level (58.66 ± 2.60 g/dl), WBC's count (14.83 ± 0.60 10^9/l) and platelets number (562.66 ± 3.17 10^9/l).

Immunity

Sesame oil has shown maximum increase (Fig. 7) in immunity of rabbits (Table 5) at 0.3ml/kg BW concentration with mean antibody titer value of 6024.66 ± 85.10 during whole experiment. Clove oil has displayed an increase in immunity (Fig. 8) at 0.2ml/kg BW concentration (Table 6) with mean antibody titer value of 5166.00 ± 140.26. The SO + CO has enhanced the immunity (Fig. 9) at 0.3+0.3 ml/kg BW concentrations (Table 7) with the mean antibody titer value of 6425.00 ± 87.36 to premier level than other groups.

Table 1. Effect of Sesame Oil on Body Weight of rabbits at 15th, 30th and 45th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	(Control)	(0.1ml/kg BW)	(0.2ml/kg BW)	(0.3ml/kg BW)
15	2.15±0.02^{a}	2.32±0.04^{b}	2.54±0.02^{c}	2.62±0.01^{d}
30	2.90±0.02^{a}	3.35±0.03^{b}	3.58±0.04^{c}	3.78±0.02^{d}
45	3.54±0.02^{a}	4.35±0.03^{b}	4.64±0.04^{c}	4.84±0.02^{d}

Means with different superscript in a row are significantly different from one another (p≤0.05) Tukey's Test

Table 2. Effect of Clove Oil on Body Weight of rabbits at 15th, 30th and 45th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	T_0 (Control)	(0.1ml/kg BW)	(0.2ml/kg BW)	(0.3ml/kg BW)
15	2.15±0.02^{a}	2.30±0.02^{c}	2.54±0.04^{d}	2.43±0.03^{b}
30	2.90±0.02^{a}	3.51±0.02^{c}	3.71±0.01^{d}	3.43±0.01^{b}
45	3.54±0.02^{a}	4.20±0.02^{c}	4.35±0.00^{d}	3.90±0.04^{b}

Means with different superscript in a row are significantly different from one another (p≤0.05) Tukey's Test

Table 3. Effects of Sesame Oil + Clove Oil on Body Weight of rabbits at 15th, 30th and 45th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	(Control)	(0.1+0.1ml/kg BW)	(0.2+0.2ml/kg BW)	(0.3+0.3ml/kg BW)
15	2.15±0.02[a]	2.49±0.05[b]	2.72±0.04[c]	3.00±0.08[d]
30	2.90±0.02[a]	3.93±0.04[b]	4.30±0.02[c]	4.61±0.07[d]
45	3.54±0.02[a]	4.69±0.02[b]	4.89±0.05[c]	5.45±0.01[d]

Means with different superscript in a row are significantly different from one another (p≤0.05) Tukey's Test

Table 4. Maximum Effect of Sesame Oil, Clove Oil, Sesame Oil + Clove Oil on Blood Parameters of Rabbits at following Concentrations with 15th, 30th and 45th Days duration

Parameters	Duration	Max Effect at following Conc. of Oils			
			Sesame Oil	Clove Oil	SO + CO
		Control	0.3ml/kg BW	0.2ml/kg BW	.3+0.3ml/kg BW
Hb (g/dL)	15th Day	10.50±0.28[a]	13.58±0.30[c]	12.06±0.08[b]	13.33±0.42[d]
RBCs (x103/µl)		5.70±0.05[a]	6.73±0.12[d]	6.13±0.18[c]	7.13±0.38[d]
HCT (%)		41.00±0.57[a]	45.33±0.33[c]	42.00±1.52[c]	48.66±0.33[d]
MCV (fL)		61.00±0.57[a]	66.00±0.57[c]	67.00±1.15[b]	68.00±0.57[c]
MCH (pg)		18.00±0.57[a]	23.33±1.20[d]	25.66±0.88[d]	31.33±1.76[d]
MCHC (g/dL)		30.00±0.57[a]	34.66±0.66[c]	37.33±1.20[c]	40.66±1.45[c]
Platelets (x103/µl)		227.33±3.71[a]	241.33±1.85[c]	305.00±6.02[c]	376.33±8.56[d]
WBCs (x103/µl)		8.00±0.57[a,b]	8.30±0.35[b]	9.00±0.35[c]	11.16±0.21[c]
Hb (g/dL)	30th Day	11.50±0.28[a]	13.56±0.31[c]	12.93±0.26[b]	15.06±0.17[d]
RBCs (x103/µl)		5.90±0.05[a]	6.83±0.08[d]	7.06±0.34[c]	8.10±0.20[d]
HCT (%)		44.00±0.57[a]	51.00±0.57[c]	51.33±1.76[c]	52.33±0.88[d]
MCV (fL)		66.66±0.88[a]	76.00±0.57[c]	77.33±2.90[b]	86.33±1.45[c]
MCH (pg)		21.66±0.33[a]	29.66±0.88[d]	32.66±0.33[d]	37.33±0.88[d]
MCHC (g/dL)		33.00±0.57[a]	38.33±0.88[c]	40.00±1.52[c]	47.66±0.88[c]
Platelets (x103/µl)		292.66±2.60[a]	331.00±1.52[c]	378.33±6.06[c]	443.66±16.18[d]
9WBCs (x103/µl)		9.33±0.88[a,b]	10.36±0.18[b]	11.40±0.20[c]	13.93±0.26[c]
Hb (g/dL)	45th Day	12.00±0.28[a]	14.25±0.38[c]	14.06±0.46[b]	16.03±0.14[d]
RBCs (x103/µ)		6.36±0.08[a]	8.06±0.12[d]	7.83±0.03[c]	9.76±0.14[d]
HCT (%)		47.00±0.57[a]	53.66±0.88[c]	53.00±2.30[c]	58.66±1.76[d]
MCV (fL)		76.66±1.45[a]	83.66±0.88[c]	83.66±0.88[b]	95.33±1.76[c]
MCH (pg)		26.33±0.88[a]	41.00±1.52[d]	36.66±0.33[d]	47.66±0.88[d]
MCHC (g/dL)		35.00±0.57[a]	46.33±0.88[c]	41.33±1.20[c]	58.66±2.60[c]
Platelets (x103/µl)		323.00±4.35[a]	342.33±0.88[c]	392.00±3.51[c]	562.66±3.17[d]
WBCs (x103/µl)		11.00±0.57[a,b]	11.86±0.13[b]	13.43±0.23[c]	14.83±0.60[c]

Means with different superscript in a row are significantly different from one another (p≤0.05) Tukey's Test

Table 5. Effect of Sesame Oil on Antibody Titer Level against Rabbit Hemorrhagic Disease Virus (RHDV) of Rabbits at 15th, 30th and 45th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	(Control)	(0.1ml/kg BW)	(0.2ml/kg BW)	(0.3ml/kg BW)
15	3220.66±82.84[a]	4091.33±112.29[a]	4458.00±142.12[b]	4858.00±142.12[c]
30	3760.66±32.05[a]	4350.00±79.99[a]	4658.00±199.58[b]	5124.66±142.16[c]
45	4105.66±34.91[a]	4588.33±54.65[a]	5258.00±142.12[b]	6024.66±85.10[c]

Means with different superscript in a row are significantly different from one another (p≤0.05) Tukey's Test

Table 6. Effect of Clove Oil on Antibody Titer Level against Rabbit Hemorrhagic Disease Virus (RHDV) of Rabbits at 15th, 30th and 45th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	(Control)	(0.1ml/kg BW)	(0.2ml/kg BW)	(0.3ml/kg BW)
15	3220.66±82.84[a]	4026.33±34.93[b]	4222.33±37.33[c]	4556.66±88.63[c]
30	3760.66±32.05[a]	4328.33±88.59[b]	4791.66±60.06[c]	4563.00±34.00[c]
45	4105.66±34.91[a]	4493.00±54.58[b]	5166.00±140.26[c]	4993.33±253.66[c]

Means with different superscript in a row are significantly different from one another (p≤0.05) Tukey's Test

Table 7. Effects of Sesame Oil + Clove Oil on Antibody Titer Level against Rabbit Hemorrhagic Disease Virus (RHDV) of Rabbits at 15th, 30th and 45th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	(Control)	(0.1+0.1ml/kg BW)	(0.2+0.2ml/kg BW)	(0.3+0.3ml/kg BW)
15	3220.66±82.84[a]	4360.00±87.57[b]	4756.33±88.95[c]	5123.33±87.31[d]
30	3760.66±32.05[a]	4597.33±115.75[b]	5225.00±112.60[c]	5759.66±91.78[d]
45	4105.66±34.91[a]	4930.33±145.64[b]	5592.00±117.24[c]	6425.00±87.36[d]

Means with different superscript in a row are significantly different from one another (p≤0.05) Tukey's Test

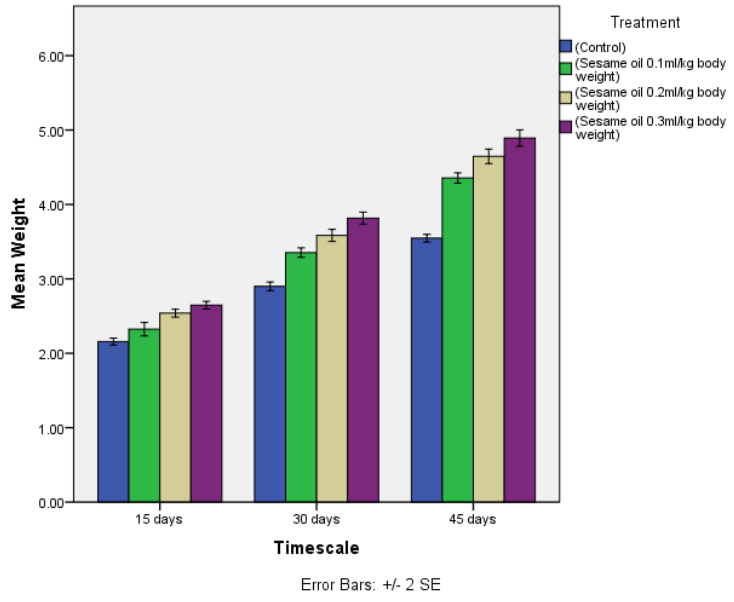

Figure 1. Concentration and Time Dependent Effect of Sesame Oil on Body Weight of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

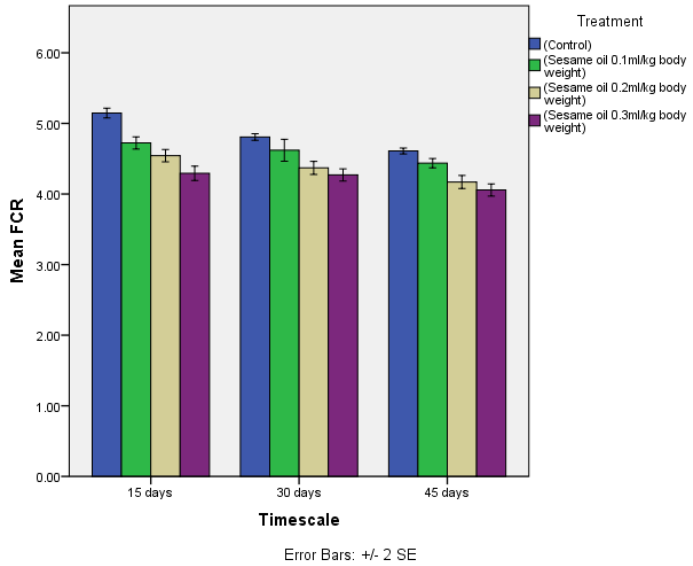

Figure 2. Concentration and Time Dependent Effect of Sesame Oil on Feed Conversion Ratio (FCR) of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

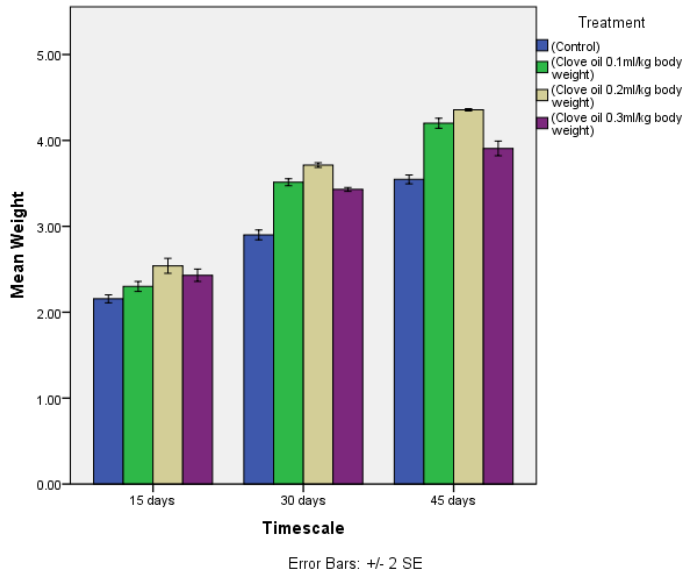

Figure 3. Concentration and Time Dependent Effect of Clove Oil on Body Weight of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

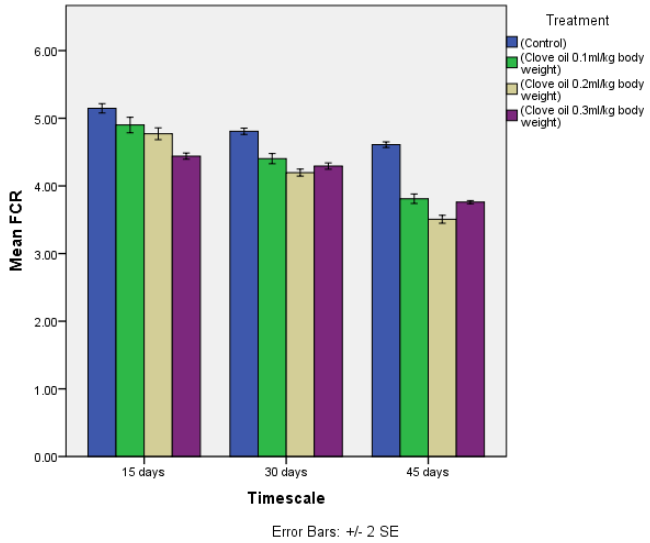

Figure 4. Concentration and Time Dependent Effect of Clove Oil on Feed Conversion Ratio (FCR) of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

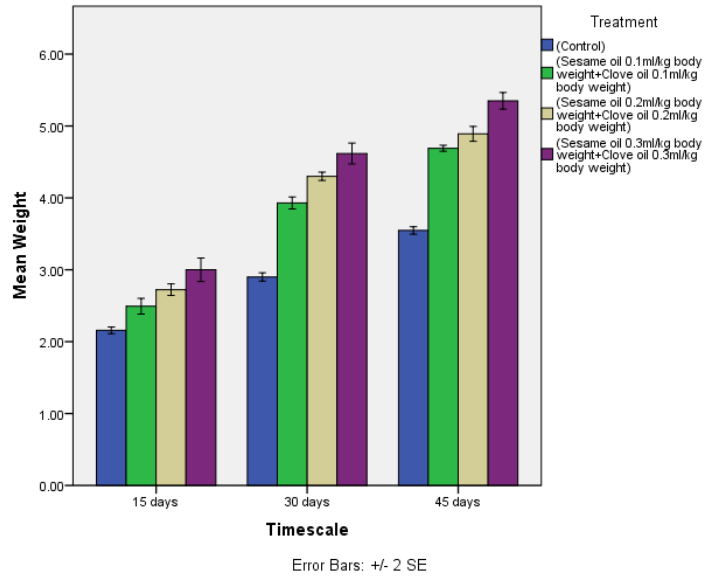

Figure 5. Concentration and Time Dependent Effects of Sesame Oil + Clove Oil on Body Weight of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

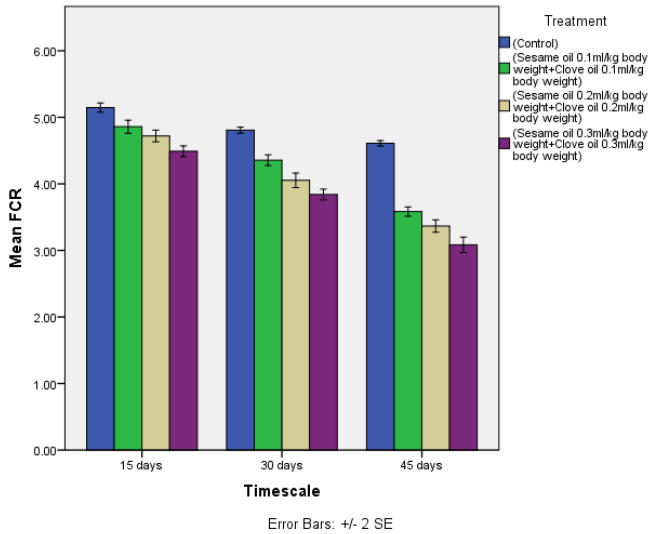

Figure 6. Concentration and Time Dependent Effects of Sesame Oil + Clove Oil on Feed Conversion Ratio (FCR) of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

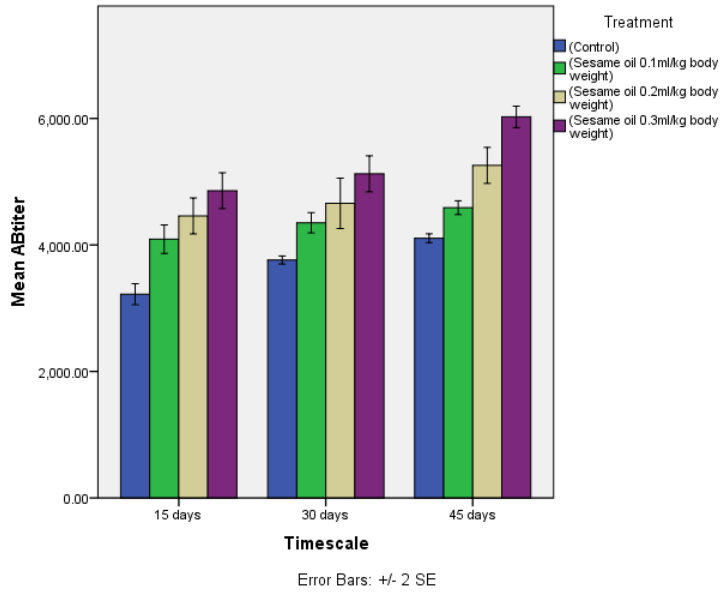

Figure 7. Concentration and Time Dependent Effect of Sesame Oil on Antibody Titer level against Rabbit Hemorrhagic Disease Virus (RHDV) of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

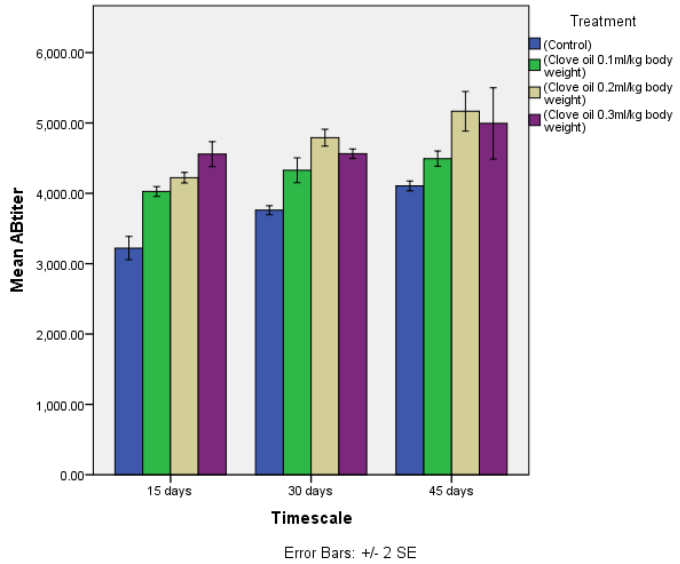

Figure 8. Concentration and Time Dependent Effect of Clove Oil on Antibody Titer level against Rabbit Hemorrhagic Disease Virus (RHDV) of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

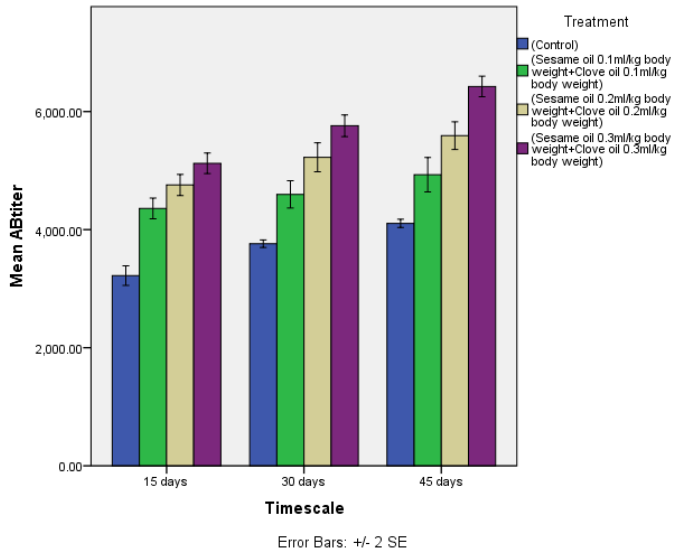

Figure 9. Concentration and Time Dependent Effects of Sesame Oil + Clove Oil on Antibody Titer level against Rabbit Hemorrhagic Disease Virus (RHDV) of Rabbits at Different Time-Scales (15th, 30th and 45th Days)

Discussion

A number of studies correlate with my findings to evaluate the effect of Sesame oil. It has been observed that Sesame oil proceeded as a good substitute of protein source with 30% amount of feed for growing rabbits and significantly increased body weight [36]. It was also indicated that Sesame oil has increased the blood constituents as red blood cells and white blood cells at dose concentrations 0.5ml, 1ml and 2ml in male rats [37]. In another study, it has revealed that Sesame oil showed beneficial influence on blood profile including blood glucose and glycosylated hemoglobin in rats with diet containing 2ml of oil [38]. In an experiment,

it has been showed that Sesame oil with 20% of feed intake has showed a protective effect in the brain to enhance the immunity of rats against the oxidative stress [39].

Similarly, the results of my work match with various studies to indicate the effects of Clove oil. It was observed that Clove oil has enhanced the feed intake and body weight gain as a natural growth promoter and raised the antibody titer level in broiler chickens at dose concentrations 0.15ml, 0.3ml and 0.45ml [40]. In another study, Clove oil has improved the fitness status, meat quality, blood components and reduced the intestinal pathogens in quails at different concentrations (0.75ml and 1.5ml/kg) [41]. It has been identified that Clove oil has improved the immune response and activated relaxation signal pathway for more immunity enhancement in rats [42]. It was also observed that dietary supplements with 0.5%, 1% and 1.5% of clove oil were useful for growth, disease resistance and strong immunity in Nile tilapia [43].

The combined use of the Sesame oil and Clove oil has also shown better results. The weight gain, blood profile and immunity has extended to high level by oils effects collectively, than separate nutrient did.

Conclusion

Sesame oil and Clove oil have significantly increased ($p<0.05$) the growth performance, blood profile and immunity of male rabbits. The addition of these oils in the diet of rabbits stimulated their digestive activity and resulted as a growth promoter. However, the specific concentration of both oils showed the positive result as; sesame oil promoted the weight, hematological parameters and immunity at 0.3ml/kg dose concentration, while clove oil enhanced the weight, blood components and immunity at 0.2ml/kg dose concentration. Sesame oil was more active than clove oil in enhancing the parameters during the whole experiment. Furthermore, SO + CO at concentrations 0.3+0.3ml/kg body weight showed the better results in improving the health of male rabbits.

Authors' contributions

Conceived and designed the experiments: M Iqbal & R Iqbal, Performed the experiments: M Iqbal, Analyzed the data: M Hussain, A Razaq, MF Malik & M Hussain, Contributed reagents/ materials/ analysis tools: M Iqbal & R Iqbal, Wrote the paper: M Iqbal & R Iqbal.

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