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Original Article

Effects of Fenugreek (*Trigonella foenum-graecum*) Seeds Saponin on Digestibility, N-Retention, Hematological parameters and blood Metabolites in Rabbits

Abdelnasir Mohmmed Ahmed Fadel Elseed[1], Tongun Danil[1], Balgees[1] Abu ElgaimAtta Elmanan[1] and Osama Hassan Ali[2]

[1]*Department of Animal Production, Faculty of Animal Production, University of Khartoum, Sudan*
[2]*Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum, Sudan*
Corresponding author's email: nasir.fadel@gmail.com

ABSTRACT

Twelve rabbits were used to examine the effect of dietary saponin of fenugreek (*Trigonella foenum-graecum*) seeds on voluntary feed intake, digestibility, N-retention and some blood metabolites. Animals were randomly assigned to three different treatments (0, 0.5 and 1 gram of saponins extract per day) with four animals per treatment. Before feeding, the different levels of fenugreek saponins extract were fed at 9:00 am, mixed with small quantities of carrot and bananas daily for a period of six weeks. Voluntary feed intake, dry matter digestibility, and live body weight gain significantly ($P<0.05$) increased in rabbits fed 0.5 and 1 gram fenugreek saponins extract. The N-retention improved in rabbits consumed 0.5 and 1 gram fenugreek saponins extract compared with the control. In contrast, cholesterol and triglyceride showed a significant reduction in rabbits ($p<0.05$) consumed the fenugreek saponions extract (0.5 and 1 gram) compared to the control. Hemoglobin concentration and red blood cell count significantly ($p<0.05$) increased in rabbits fed fenugreek saponins extract compared to the control. Inclusion of fenugreek saponins extract to the diet concluded positive effects on production, hematological parameters and blood Metabolites of rabbits.

Key words: Fenugreek Seeds Saponin, Digestibility, Blood Metabolites, Rabbit

INTRODUCTION

The domestic rabbits are used for many purpose and they are an important source of food. The acceptability of rabbit as a farm animal worldwide is due to its comparative advantage over livestock species that suggested the increase in its production can cover the gap in animal protein requirement for human consumption especially for developing countries. Rabbit convert locally available plant, by-product and grass into animal protein (Ramchurn et al. 2000) and they efficiently used to make a plant (Timon and Hamrahan, 1985).

Animal feed additives are pharmaceutical or nutritional substances that are not natural feedstuffs and added for various purposes, such as control of infectious diseases in animals, growth promotion and enhancing feed digestibility in animals (Francis et.al. 2005 and Henry and Alexis, 2009). Raghuram *et al.* (1994) reported that Fenugreek seed is an important source of steroidal saponins such as diosgenin which are used extensively by both pharmaceutical and nutritional industries. Saponins are common in a large number of plants and plant products and playing an important role in human and animal nutrition. Saponins have biological role as membrane-permeabilizing, immunostimulant and hypocholesterolemic properties and it was found to have significant effects on growth and feed intake in animals. These compounds have been observed to improve the protein digestion and the uptake of vitamins and minerals in gut and act as hypoglycemic agent (Francis et al., 2002). However, animal nutritionists have generally considered saponin to be deleterious compounds.

This study aimed to examine the effect of feungreek (*Trigonella foenum-graecum*) saponins on digestibility, urinary nitrogen retention and some blood metabolites.

MATERIALS AND METHODS

Saponins Extraction

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Fenugreek *(Trigonella foenum-graecum)* seeds were sun dried, grinded and defatted by using Soxhelt Apparatus 150 ml of Petrelum Ether at 70 to 80 C° for 6 hours to remove of fat and then extraction was performed with Ethanol (Extraction Solvent 7%) for 8 hours.

Experimental animals

The study was carried out in the experimental unit, Department of Animal Nutrition, Faculty of Animal Production, University of Khartoum. Twelve rabbits with average body weight of 1.2 ± 0.15 Kg were used in this study. All animals were domestic with predominant colors: White, Brown, Grey and Black. Feeding adaptation of animals was performed in a period of two weeks then distributed randomly to the cages and provided with food and water *ad-libitum*.

Housing

The battery cages were made from Iron sheet and wire mesh, it is over half meter brick, each block of battery cages is divided into pens with dimensions of length 120 cm, width 60 cm and height 60 cm, and self-cleaning of wire floor and a tray under mesh. They were provided with waterer and feeders fastened to the floor.

Experimental treatments

Experimental rations (Tables 1 and 2) were formulated to meet the nutrient requirements of rabbit (NRC, 1977). Fresh alfalfa was provided twice a week as roughage. Animals were randomly assigned to one of three different treatments (0, 0.5 and 1 gram of saponins extract per day) with four animals per treatment following the completely randomized design. Before feeding, the different levels of fenugreek saponins extract were fed at 9:00 am a mixed of small quantities of carrot and banana were offered.

Table 1. Ingredient composition of the experimental ration

Items	%
Sorghum	36
Groundnut Cake	4
Wheat Bran	56
Dicalcium Phosphate	2.4
Muilti-Vitamins	1.6

Table 2. Chemical analysis of experimental feed

Items	%
Dry matter	94.76
Crude protein	33.33
Crude fiber	24.51
Ether extract	4.140
Ash	5.340
N- free extract	26.44
Organic matter	89.42

Feeding Trial

Animals were weighed on the first day of the experiment as initial weight, and then systematically weighed every week until the end of experimental period, where the final weight was recorded. Feed intake was determined weekly for each individual animal.

Collection of samples

At the end of each week for four weeks after two week adaptation period, 5 ml blood samples were withdrawn from the jugular vein of rabbits and kept in 2 separate test tubes (3 ml in plane vacutainer tube and 2 ml in heparinized vacutainer tube). The sera were harvested and preserved at -20C° for subsequent assay of serum cholesterol, triglycerides and minerals (Na, Ca, and K). In addition, red blood cell count (RBC count), differential leucocytes count and hemoglobin concentration (Hb) were determined by using whole blood samples collected in the heparinized vacutainer tubes. Feces and urine samples were collected and analyzed for their Nitrogen contents.

Estimation of serum total cholesterols and triglycerides:

Serum cholesterol concentration was determined by an enzymatic-spectrophotometric method (Allain et al. 1974; Sevnsson 1982 and Fossati and Prencipe 1982). The Cholesterols and Triglycerides of samples were determined by means of the coupled reaction, a coloured complex which can be measured by spectrophotometry**.**

Calcium, Sodium and Potassium determination

The concentration of Ca, Na and K in the plasma was determined according to the colorimetric methods (Trinder, 1960; Wootton, 1974).

Hematological analysis

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Differential leucocytes count was carried out by using blood smear stain with Giemsa stains. Red blood cells count was performed in an improved Neubauer haemocytometer. Hemoglobin concentration was determined by cyanmethaemoglobin method as described by Van Kampen and Zijlstra (1961).

Feces and urine analysis

Feces and urine samples were used for determination of dry matter (DM), crude protein (CP) and kjeldahl-Nitrogen according to AOAC (1990).

Statistical analysis

The data obtained from feed intake, milk yield and composition, and serum for hormone were subjected to Statistical analysis of variance (ANOVA) for completely randomized design using computerized program known as statistix[8]. A least significant difference (LSD) was carried out to test significant difference between the treatment means.

RESULTS AND DISCUSSION

Live Weight

The biological performance of the rabbits is given in figure 1. At the beginning of the feeding trial (30 days), live body weights were similar, but final live body weight were significantly ($p<0.05$) higher in the groups fed with the two levels of fenugreek *(Trigonella foenum-graecum)* saponin 0.5 (T_1) and 1 gm., (T_2) from first week to fourth week. In comparison to the control group, the higher weight was recorded at week 3 in group T_2

Digestibility

Table 3 shows the effects of fenugreek **(***Trigonella foenum-graecum*) saponin on digestibility of the dry matter and crude protein. Addition of fenugreek **(***Trigonella foenum-graecum*) saponin resulted a significant difference ($P\leq 0.05$) in the Dry matter digestibility, but not in the crude protein digestibility. However, there was a numerical decrease in crude protein digestibility. The fenugreek *(Trigonella foenum-graecum)* saponin treatment groups (T_1 and T_2) exhibited significant increase in voluntary feed intake. This is in agreement with the observation of Diaz *et al.* (1994) who reported that supplementation of *sapindus saponaria* saponin in sheep inhibits rumen protozoa and increases the population of the bacteria and fungi and consequently dry matter digestibility. The significant increase in the dry matter digestibility resulting from incorporated fenugreek saponin might be associated with an improved pattern of caecal fermentation; this effect is likely to be associated with the selective effect of saponin on bacteria/protozoa ratio, adaptation and quantity.

The numerical decrease in crude protein digestibility could be attributed to formation of sapringly digestible saponin- protein complex due to the lower prtoeolytic enzyme activity in the ceacum.

Voluntary Feed Intake and Weight Gain

The average means of voluntary feed intake and body weight gain are shown in Table 3 and figure 1; there were significant ($P\leq 0.05$) increases in both body weight gain and voluntary feed intake in response to addition of different levels of fenugreek saponin. Noteworthy, the highest body weight gain was recorded in 1 gm fenugreek-treated group, while, the lowest body weight gain was noticed in rabbit fed control diet. This clearly indicated that fenugreek saponin has a positive effect on feed intake and body weight gain. Consistently, Podgorsik and Majewski (2002) found that saponin increases feed intake and water intake in broiler chicks, which are attributed to anti-oxidant activities of saponin leading to increase in digestive enzymes and decrease in bacterial activities. Normally, rats consume 80-90% of their feed intake at night; nevertheless, steroid saponin-rich fenugreek seed extract affects their circadian rhythm of feeding behavior (Petit *et al.,* 1995). The feed consumption has been found to be stable throughout the day in saponin ingested group resulted in an increase in voluntary feed intake and body weight gain (Petit *et al.,* 1995).

However, these results disagree with Kaya *et al.* (2006) who did not observe any improvement in feed intake and body weight gain in lambs fed saponin. These differences in response to saponin could be attributed to differences in acceptance of saponin by these species and might be related to sensitivity to saponin content or due to structure differences in their sapogenin fraction. Nevertheless, the present data demonstrated that inclusion of fenugreek *(Trigonella foenum-graecum)* saponin had an effective increase in voluntary feed intake and improvement of digestibility and consequently stimulating growth.

Nitrogen Balance

The effect of fenugreek **(**saponin on Nitrogen balance is shown in Table 3, inclusion of fenugreek saponin (0.5 g and 1 g) significantly ($P\leq 0.05$) increased Nitrogen intake but did not affect urinary nitrogen, faecal nitrogen and consequently, Nitrogen retention. These findings agreed with those of Leng and Nolan (1984) who noted that 50– 80% of the Nitrogen requirement resulted from ruminal-Nitrogen pool food degradation and microbial lysis. The plant extracts improved efficiency of Nitrogen (N) utilization in the rumen, which attributed to the binding of ammonia by plant extracts when the ruminal ammonia concentration is high and release of the bound ammonia when its concentration is low (Makkar *et al.,* 1998). Thus, modulating diurnal fluctuations in ruminal ammonia concentrations to provide a continuously adequate of ammonia for microbial metabolism. Higher microbial mass, lower proportion to short chain

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fatty acid (SCFA) and lower gas production in the *in vitro* fermentation system lead to the same or higher true digestibility of substrate in the presence of plant extracts (Makkar *et al.*, 1998).

The significant increase in Nitrogen intake in the present study could be attributed to addition of fenugreek saponin, which increased protein degradation and part of microbial lysis, in addition to high dry matter intake.

Effect of fenugreek saponin on hematological parameters

Effects of fenugreek saponin on blood and serum constituents of rabbits are shown in figures (2-13). Rabbit groups supplemented with 1 g of feungreek saponin showed significant ($P \leq 0.05$) decrease in serum cholesterol and triglycerides level compared to the control group Figures 2 and 3. This decrease was highly significant after supplementation of feungreek saponin for four weeks. This finding agreed with that of Szkudelski *et al.* (1998) and Atta Elmnan and Mangara (2012) who reported that reduction in cholesterol may be attributed to the effect of saponin present in fenugreek seed and improved serum lipid profile with significant reduction in serum total cholesterol. Moreover, similar result demonstrated by Raju *et al.* (2001) demonstrated that fenugreek saponin decreases the low density lipoprotein (LDL) and the very low density lipoprotein (VLDL) cholesterol and triglycerides. Fenugreek saponin increases biliary cholesterol excretion in the liver and inhibits cholesterol and triglycerides absorption and synthesis (Al-Habori and Raman (1998).

The results in figures 4 and 5 show significant differences ($p<0.05$) in hemoglobin concentration and red blood cells count. This finding correlates with that of (Mogha *et al.*, 2012), who reported that extract of fenugreek may be an important factor for increasing the biosynthesis of hemoglobin and raises blood level due to present of saponin.

The current findings show significant increases ($p<0.05$) in serum Na, K and Ca concentrations and this increase reach the peak after 2 weeks of Fenugreek saponin ingestion (Figure 6-8). Noteworthy, the aqueous and benzene extract of fenugreek has been found to show diuretic activity in a dose-dependent manner by increasing the volume of urine and natriuretic activity due to increasing the levels of Na ions ratio in Wistar rats (Rohini *et al.*, 2009); which can be employed to treat hypertension.

The present data showed Significant differences ($p<0.05$) in lymphocyte, neutrophil, eosinophil, monocyte and highly significant difference ($p<0.05$) in basophil ratios in response to saponin ingestion (Figures 9-13). Saponins induce a strong adjuvant effect on T-dependent as well as T-independent antigens responses; they also induce strong cytotoxic CD8+ lymphocyte responses and potentiate the response to mucosal antigens (Kensil, 1996). The mechanisms of immune-stimulating action of saponins have not been clearly understood; Saponins reportedly induce production of cytokines such as interleukins and interferons that might mediate their immunostimulant, anti-inflammatory, anti-bacterial and anti-parasitic effects (Kensil, 1996).

Inclusion of fenugreek saponins extract to the diet concluded positive effects on production, hematological parameters and blood Metabolites.

Table 3. Effect of Fenugreek (*Trigonella foenum-graecum*) Saponin on performance and N- balance in rabbit

Items	Control	Saponin levels		SEM	Significance Level
		0.5 gm	1 gm		
Crude Protein Digestibility	88.08	83.42	84.04	3.76	**NS**
Dry Matter Digestibility	83.25	85.92	87.46	0.17	*
N- Intake (g/d)	2.54	2.99	3.08	0.06	*
Urinary nitrogen(g/d)	0.47	0.68	0.58	0.09	**NS**
Fecal nitrogen(g/d)	0.55	0.50	0.49	0.09	**NS**
Nitrogen retention (g/d)	1.51	1.81	2.01	0.20	**NS**
Voluntary feed intake(g/d)	47.58	56.16	57.81	1.20	*
Body weight Gain(g/d)	6.24	7.70	11.55	1.27	**

*=P<0.05; **= P<0.01; NS=Not Significant; SEM=Standard Error of Means

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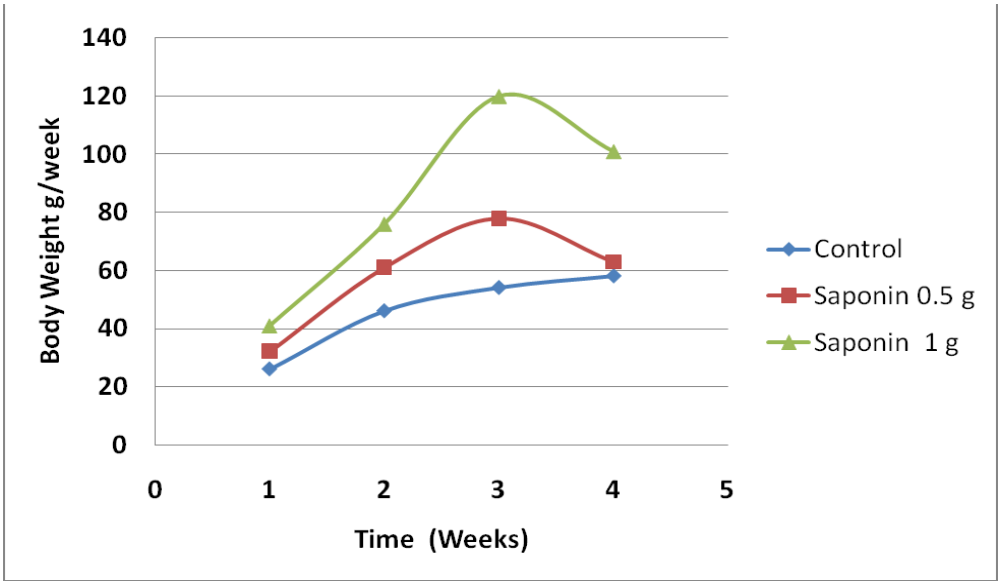

Figure 1. Effect of fenugreek (*Trigonella foenum-graecum*) saponin on live body weight in local rabbits (g)

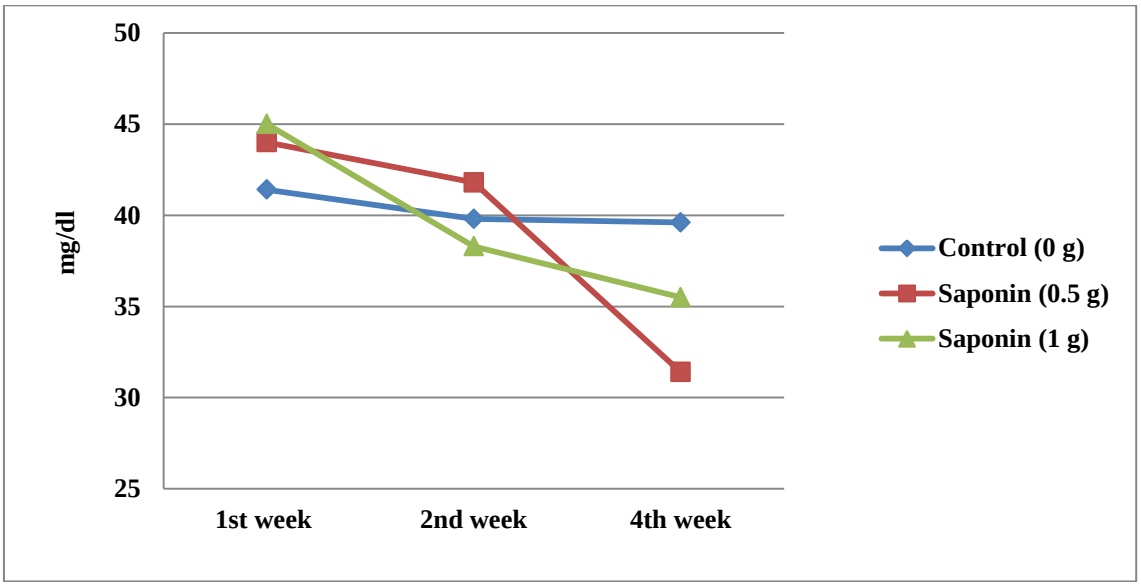

Figure 2. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on serum cholesterol concentration (mg/dl) in rabbits

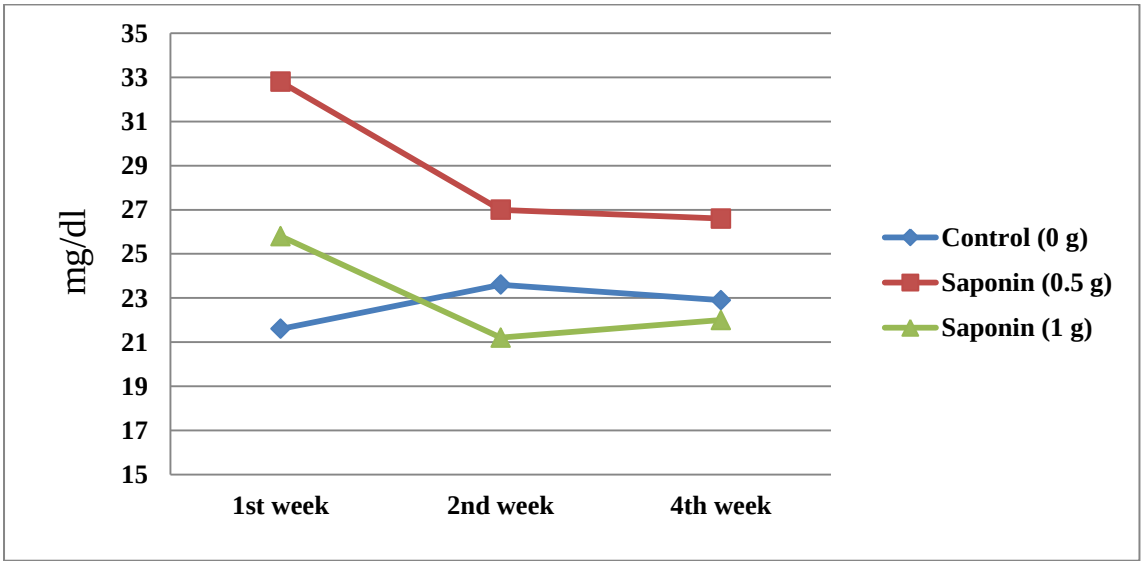

Figure 3. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on serum Triglycerides concentration (mg/dl) in rabbits

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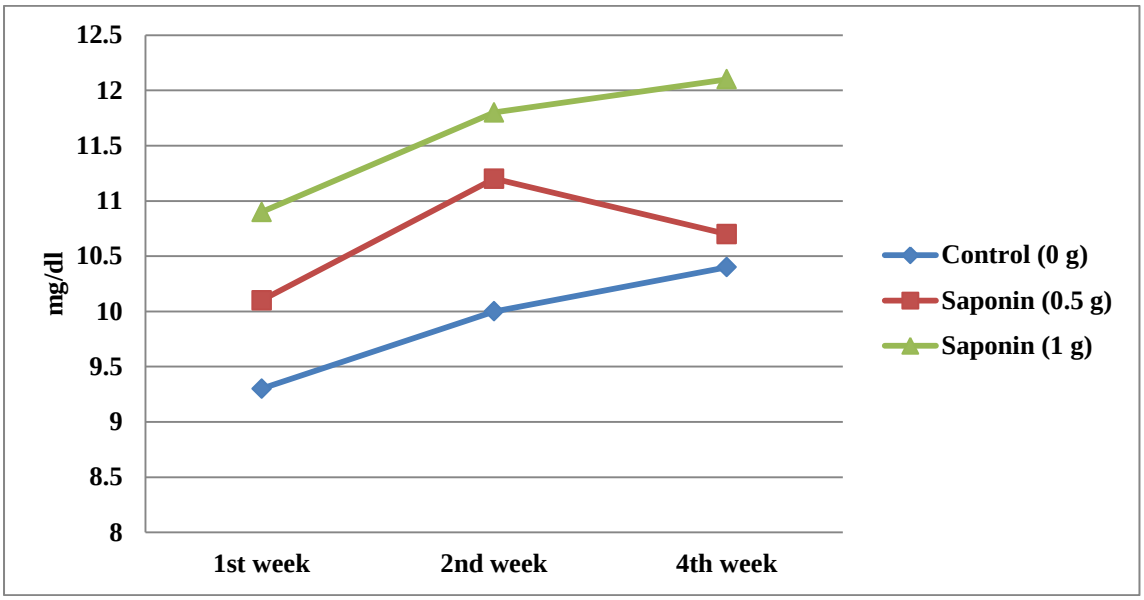

Figure 4. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on Blood Hemoglobin concentration (mg/dl) in rabbits

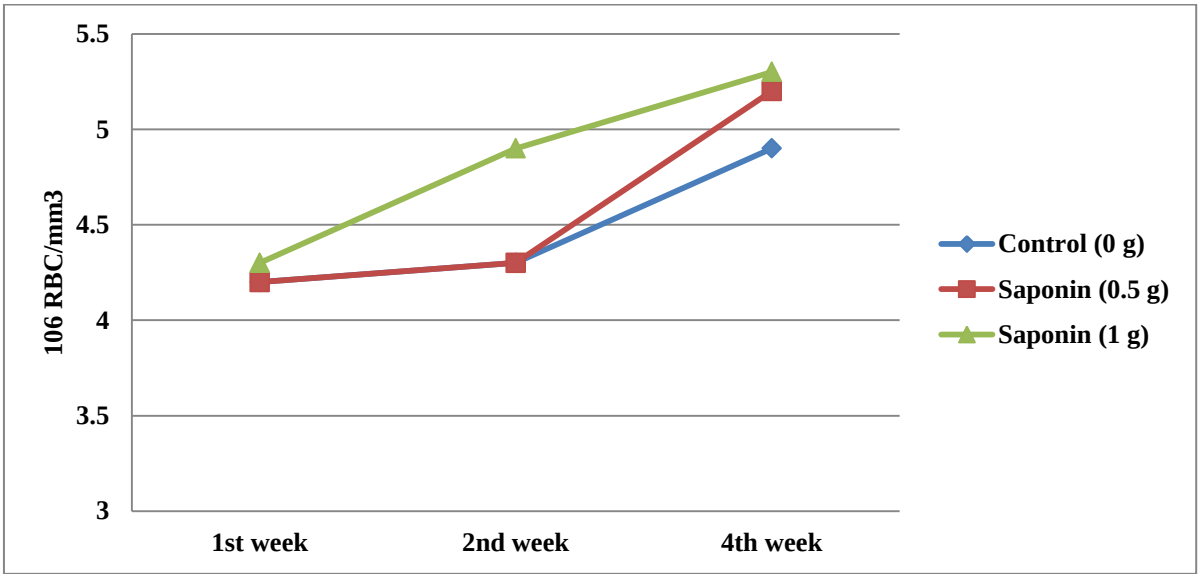

Figure 5. Effect of Fenugreek (*Trigonella foenum- graecum*) Saponin on red blood cells count in rabbits

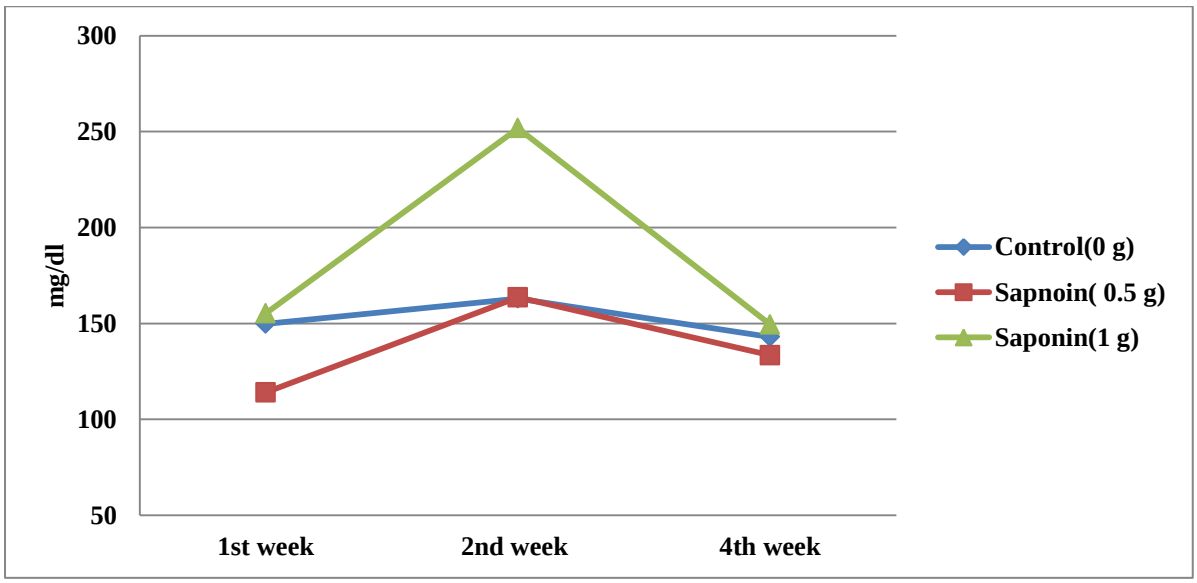

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Figure (6). Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on serum sodium concentration (mg/dl) of rabbits

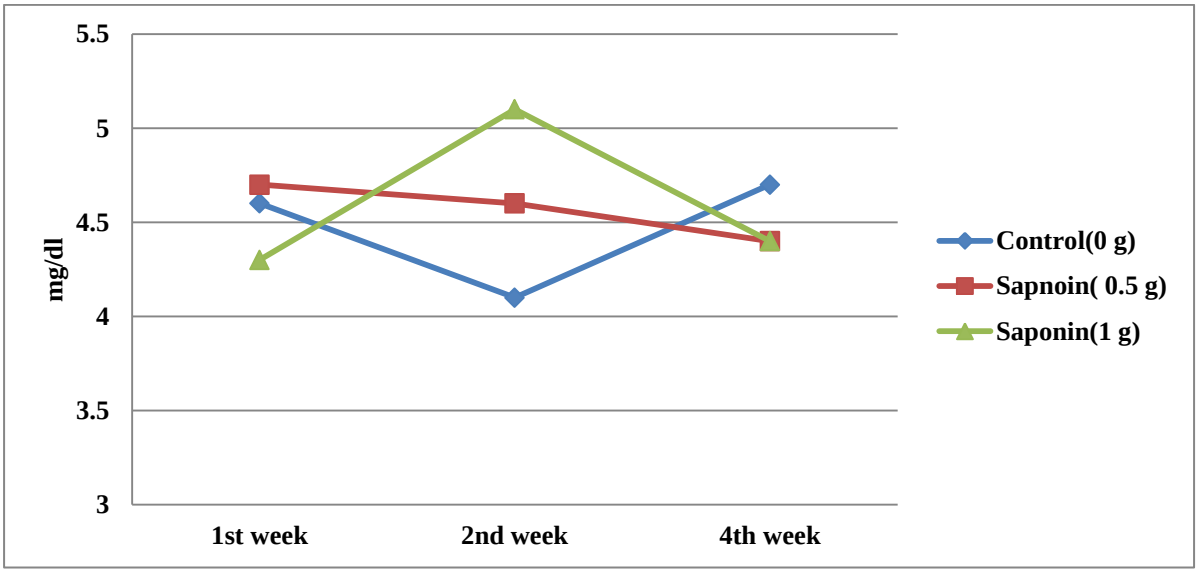

Figure 7. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on serum potassium concentration (mg/dl) in rabbits

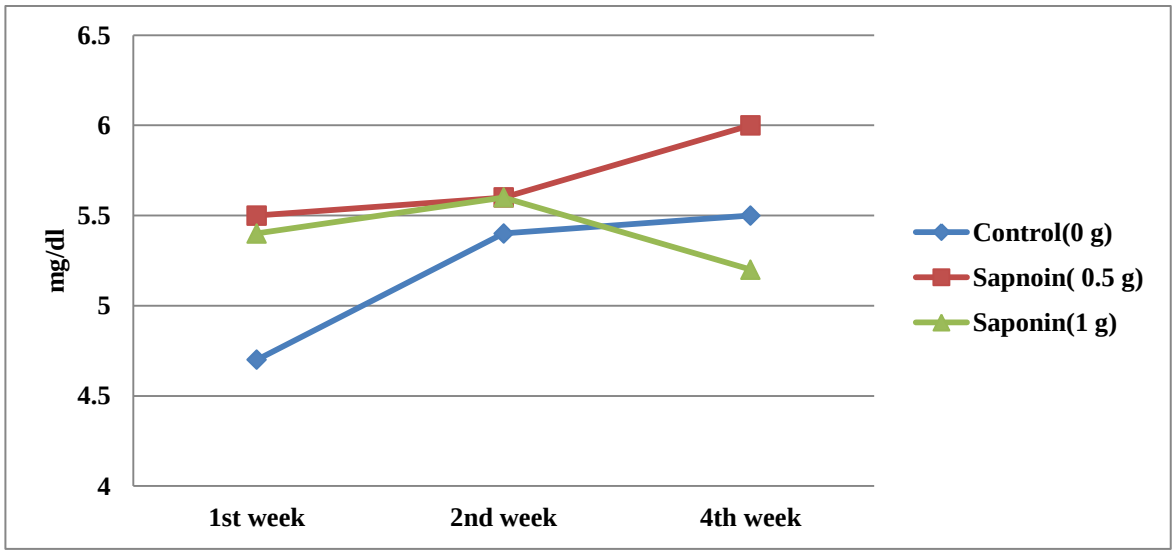

Figure 8. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on serum calcium concentration (mg/dl) in rabbits

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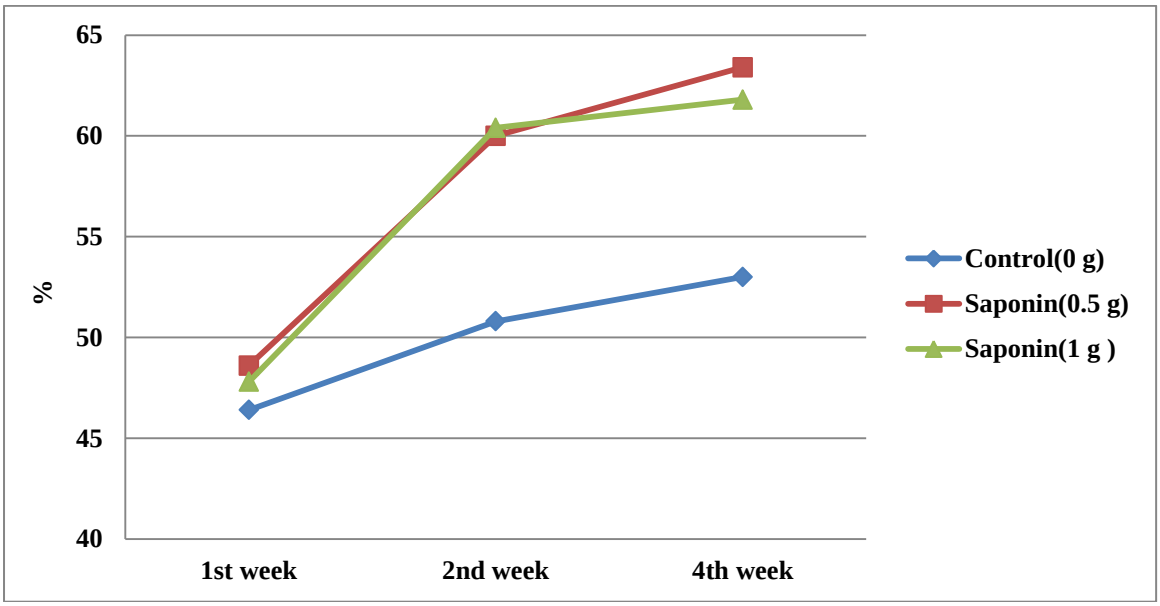

Figure 9. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on Lymphocyte White Blood Cells percentage in rabbits

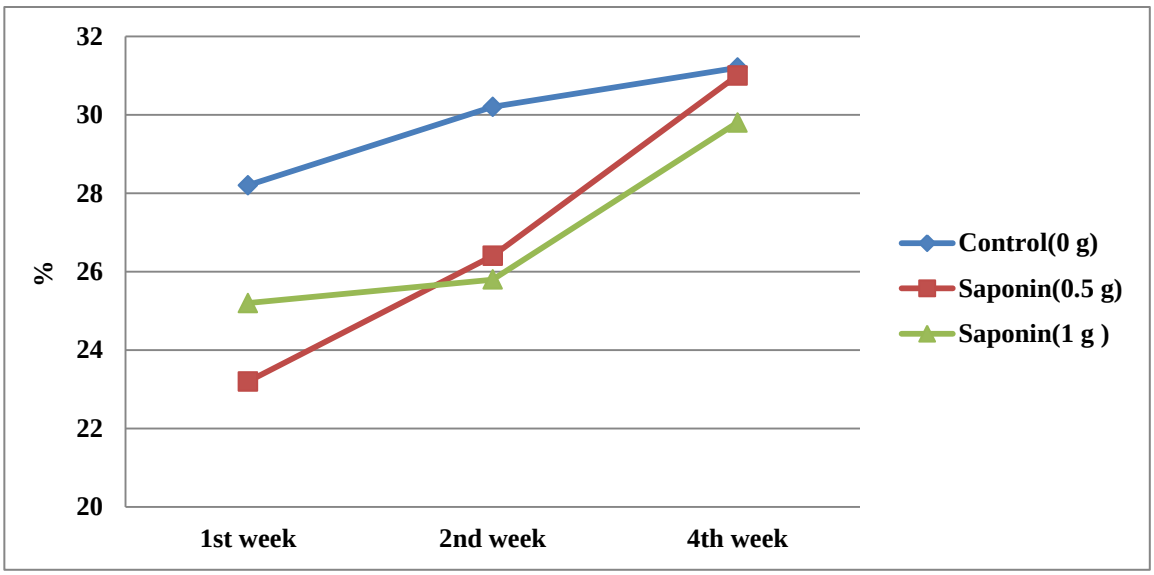

Figure 10. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on Neutropill White Blood Cells percentage in rabbits

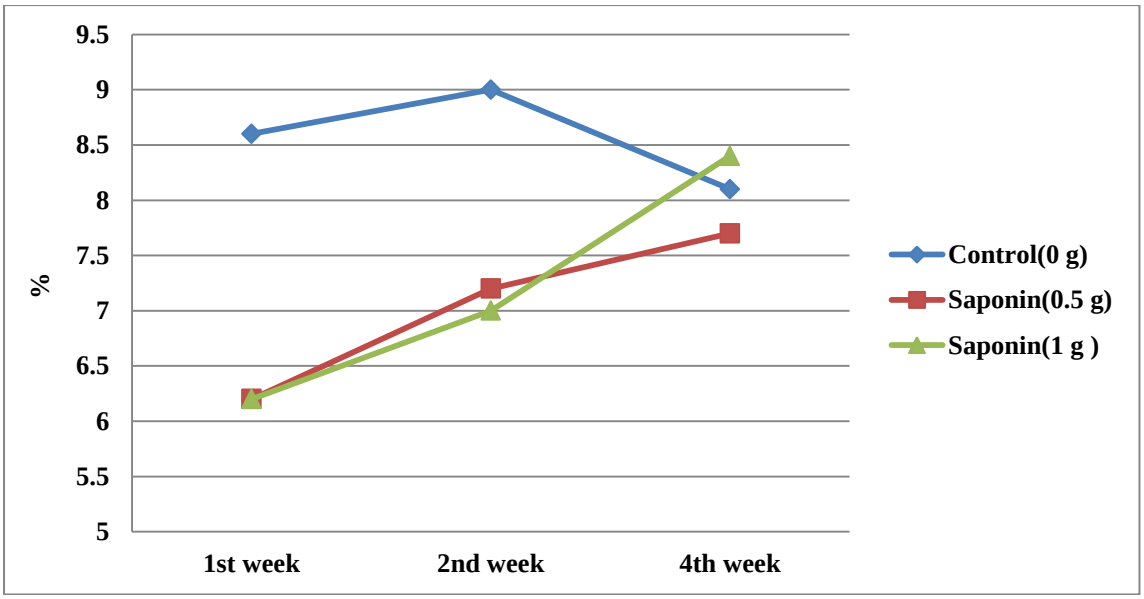

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Figure 11. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on Eosinophill White Blood Cells percentage in rabbits

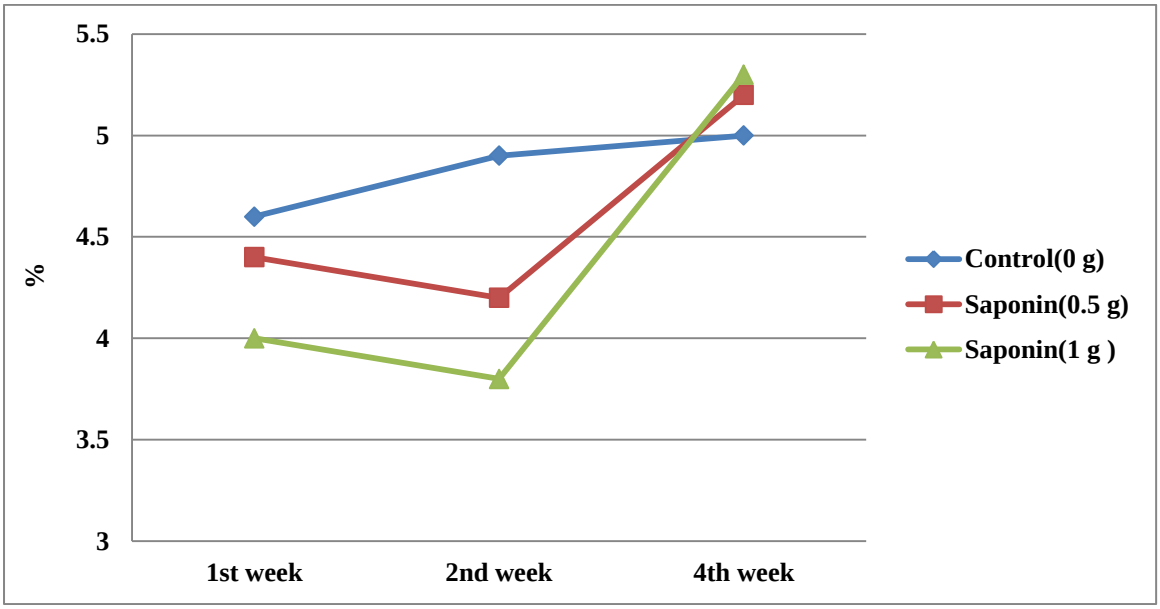

Figure 12. Effect of Fenugreek (*Trigonella foenum- graecum*) saponin on Monocyte White Blood Cell percentage in rabbits

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