Original Article

Ground *Lepidium sativum* Seeds Affect Immune System Cells, IgM Levels, Body Weights, and Hematology in Rats

Sawsan Hassan Mahassni, Kholood Khaled Nabulsi

Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Background: Garden cress seeds(*Lepidium sativum* seeds [LSS]) are widely used in traditional medicine for different effects. This study was done to determine the effects of ground LSS on the immune system, which is an effect attributed to LSS with no sufficient proof. Thus, hematology, IgM concentrations, body weights and total and percent relative total body weight gains, and feed and water intakes were determined in rats for two experimental periods. This is the first study to determine the effects of LSS in healthy subjects on water intake, counts white blood cell (WBC) types, IgM levels, and differences between two experimental periods for the measured parameters. In addition, this is the first study to use 15% LSS mixed with the diet for the studied parameters. **Materials and Methods:** Thirty-six young male Wistar albino rats were equally divided into low dose (LD) and high dose (HD) groups administered with 10% and 15%, respectively, ground LSS mixed with the regular feed (w/w) daily, and a control group administered with the regular feed. Blood samples were collected on days 30–32 and again on days 38–40 of the experiment. **Results:** Comparing the results of the LSS groups to the control: LSS led to higher body weights and total body weight gains, lower feed and water intakes for both experimental periods, lower hemoglobin and IgM levels for the LD group for the shorter experimental period only, higher total and differential WBC counts/percents, and lower lymphocytes percent for the HD group. **Conclusions:** LSS affected both humoral and innate immunities, hemoglobin concentration, and body weight with some dependence on the period of consumption.

Keywords: Body weight, hematology, immune system, IgM, *Lepidium sativum* seeds, white blood cells

Introduction

Different parts of many plants and herbs have been used by ancient traditional and alternative medical systems for the treatment of many ailments. In addition, present time modern medicine uses many medications that are based on or contain active components of some seeds, spices, plants, and herbs. The advantages of using natural components for the treatment of diseases and ailments are their availability, low cost, and few or no side effects. One such important plant is the *Lepidium sativum* Linn plant, commonly known as garden cress, which is a member of the *Brassicaceae*, or *Cruciferae,* family. Both the plant and its seeds are widely used as a food condiment and for the treatment of different diseases and conditions by traditional and folk medicinal systems. *Lepidium sativum* seeds (LSS) are very nutritious, containing proteins, carbohydrates, fatty acids, flavonoids, minerals, and vitamins.[1,2] The seeds have antibacterial, antioxidant, analgesic, anti-diarrheal, laxative, anti-asthmatic, anticancer activities, and many other effects.[1,3-7]

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LSS, used as aqueous or alcoholic extracts, oils, or ground, have been studied in tissue culture, human, and laboratory animal studies[3,8-11] for the treatment of many diseases and ailments, such as diabetes,[12] asthma,[6] cancer,[3,11] and anemia.[13] A commonly believed effect of LSS is in strengthening the immune system, which is highly sensitive to lifestyle factors and diet. A small number of studies[8,10,14-17] investigated the effects of LSS on the immune system of healthy animals, such as mice, rats, and chicks with no consensus in the findings. It is not surprising that LSS are beneficial in cases of anemia

Address for correspondence: Dr. Sawsan Hassan Mahassni, Department of Biochemistry, Faculty of Science, King Abdulaziz University, Box 42652, Jeddah 21551, Saudi Arabia.
E-mail: sawsanmahassni@hotmail.com

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since they are a rich source of nonhem iron. On the other hand, studies are contradictory regarding LSS ability to increase hemoglobin levels and red blood cell (RBC) counts in healthy animals.[8,10,17]

Studies on LSS in healthy humans or animals are very few, while more studies have been done on diseased humans[6,13] and animals.[3,11,12] Therefore, the aim of this study was to determine the effects of ground LSS, mixed with the normal rat feed at two different doses, on total and differential white blood cells (WBC) counts and percents, hematological parameters, body weights and total and percent relative total body weight gains, and feed and water intakes in healthy rats at two different experimental periods. This is the first study to determine the effects of LSS on IgM levels and the difference in hematological parameters between two experimental periods. This is done to determine if LSS do in fact affect the immune system and if the effect is dependent on a specific time period. This would help in providing better guidelines for the use of LSS in humans.

Materials and Methods

Experimental animals and groups

Thirty-six male albino Wistar rats, at 9 weeks of age and weighing 200–265 g, were used in this study. The rats were maintained at room temperature and were exposed to 8–10 h of artificial light in the daytime. Feed and water were provided *ad libitum*. The rats were placed in the laboratory 5 days before the beginning of the experiment to acclimatize to the laboratory conditions. All rats were weighed, and the consumed feed weight and water volume were measured 6 days/week for the entire experimental period.

The rats were divided equally into a control group and two experimental groups. The control group rats were given the regular feed pellets for a period of 37 days. The low dose (LD) group rats were given LSS mixed with the regular feed at a dose of 10% weight/weight (w/w) daily for a period of 39 days. Finally, the high dose (HD) group rats were given LSS mixed with the feed pellets at a dose of 15% (w/w) daily for a period of 38 days.

This study was reviewed and approved by the Ethical committee of the Biochemistry Department, King Abdulaziz University, Jeddah, Saudi Arabia. All regulations for the ethical treatment of laboratory animals and animal welfare guidelines were followed.

Preparation of the experimental diets

The LSS used in this study were grown locally in Al-Qaseem region, Saudi Arabia, and they were obtained fresh from a local herbs shop. The used laboratory animal feed (Saudi Grains Organization, Jeddah, Saudi Arabia) contained protein; crude fat; crude fiber; ash; salt; calcium; phosphorus; vitamin A, D, and E; and traces of some heavy metals. To prepare the experimental diets, the LSS and the regular laboratory animal feed pellets were ground to powder separately.[8] Two different concentrations of LSS powder (10% and 15%) were mixed separately with the animal feed powder and formed into pellets.

Collection of blood samples

Blood samples were collected from all rats, on 2 different days, through the retro-orbital sinus under diethyl ether anesthesia after an overnight fast. The blood samples were collected on days 30 and 38 of the experimental period for the control, days 32 and 40 for the LD group, and days 31 and 39 for the HD group.[14] Two rats from the HD group died on the 31st day of the experimental period during blood withdrawal. Whole blood was collected in ethylenediamine tetraacetic acid vacutainer test tubes for the differential complete blood counts (CBCs) and in vacutainer test tubes coated with silica and containing polymer gel for the separation of serum for the determination of IgM concentrations. These tubes were centrifuged at 3500 rpm for 5 min to separate the serum from the blood clot.

Calculation of mean daily body weight, total body weight gain, and percent relative total body weight gain

The mean daily body weight for each group was calculated. The mean total body weight gain for each group was calculated by subtracting the initial body weight for each rat from its final body weight and then calculating the mean for the group. The mean percent relative total body weight gain for each rat was calculated using the following equation,

% Relative total body weight gain = (final weight-initial weight/initial weight) × 100

Subsequently, the mean percent relative total body weight gain was calculated for each group.

Calculation of mean daily feed intake, water intake, and feed efficiency ratio

The daily feed intake for each group was calculated by subtracting the weight of the remaining feed from the weight of feed placed in the cages the day before. Subsequently, the mean feed intake was calculated for each group. The mean water intake for each group was calculated as done above for the feed intake, using the volume of water consumed daily. The daily body weight gain for each rat was calculated by subtracting the body weight for the previous day from the current body weight and then calculating the mean for all the rats in the group. The daily feed efficiency ratio (FER)[14] for each group was calculated using the following equation:

Daily FER = daily body weight gain for the group/daily feed intake for the group

Determination of the differential complete blood count

Total WBC and RBC counts for the first and second blood samples were counted under a microscope using a hemocytometer and Turk's solution for WBC and Hayem's solution for RBC. The differential WBC counts were determined for the second blood under a microscope using the Diff-3 Stain pack (Atlas Medical, Cambridge, UK). For the determination of hemoglobin concentrations for the first and second blood samples, the hemoglobin liquicolor

kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) was used. The optical density for the final product was measured at 546 nm using a GENESYS spectrophotometer (G10S UV-VIS, Thermo Fisher Scientific, Madison, Wisconsin, USA).

Determination of serum IgM concentration

A Rat IgM ELISA Kit (Elabscience, Wuhan, Hubei, China) was used to determine the IgM concentrations for the first blood collection. The kit was done according to the manufacturer's instructions, and the optical density for the final product was measured at 450 nm using a Synergy HT microplate reader (BioTek Instruments, Winooski, Vermont, USA). The serum IgM concentrations were calculated using a standard curve and the GraphPad Prism program (Version 6.01, GraphPad Software, San Diego, California, USA).

Descriptive and analytical statistics

Statistical analysis of the data was done using the MegaStat statistical program (Version 9.4, Butler University, Indianapolis, Indiana, USA). The tests used for the significance in the differences between the three groups were the one-way ANOVA test for the normally distributed parameters and the Kruskal–Wallis test for the non-normally distributed parameters. For the *post hoc* analysis for the unmatched groups, the *t*-test was used for the normally distributed parameters, and the Mann–Whitney test was used for the non-normally distributed parameters. For the comparisons between the matched groups, the paired *t*-test was used for the normally distributed parameters, while the Wilcoxon test was used for the non-normally distributed parameters. Differences were considered significant (S) at $P < 0.05$, highly significant at $P < 0.01$, and non-significant at $P \geq 0.05$.

Results

Body weights, feed and water intakes, and feed efficiency ratio

The results for the mean body weights and total and percent relative total body weight gains, daily feed and water intakes, and FER for the groups were compared between different experimental periods. The period from the beginning of the experiment to the collection of the first blood samples (days 30–32) is termed as the initial experimental period. The period from the beginning of the experiment to the collection of the second blood samples (days 38–40) is termed the complete experimental period. Finally, the period between the collection of the first and second blood samples is termed the final experimental period.

For the initial, final, and complete experimental periods [Table 1], the mean daily body weights and daily feed and water intakes were each significantly different between the groups. As for the mean total body weight gain, only the initial and complete experimental periods led to a significant difference. For the *post hoc* analysis [Table 2], compared to the respective controls, the mean daily body weights were significantly higher for the HD group during the initial experimental period and for both the LD and HD groups for both the final and complete experimental periods. The mean total body weight gains were significantly higher for the HD group for both the initial and complete experimental periods compared to the respective controls. The mean daily body weights for the HD group for the three periods were significantly higher than the respective mean for the LD group. The mean daily feed intakes for the initial and complete experimental periods for the LD group were

Table 1: Statistical analysis for the mean daily body weights calculations, daily feed and water intakes, and feed efficiency ratio

Parameter	Group	Initial period			Final period			Complete period		
		n	Mean±SD	*P*	*n*	Mean±SD	*P*	*n*	Mean±SD	*P*
Daily body weight (per rat, g)	Control	11	244±4.95	0.000 (HS)*	11	246±7.89	0.001 (HS)**	11	244±5.69	0.000 (HS)*
	LD	12	248±10.21		12	259±6.31		12	250±10.47	
	HD	12	263±10.92		10	275±8.10		12	266±11.46	
Total body weight gain (g)	Control	11	15±14.80	0.026 (S)*	11	20±7.94	0.659 (NS)*	11	14±16.93	0.034 (S)*
	LD	12	25±18.79		12	19±9.41		12	29±19.30	
	HD	12	33±11.63		10	18±5.31		10	34±14.60	
Percent relative total body weight gain	Control	11	6±6.20	0.052 (NS)*	11	9±3.38	0.377 (NS)*	11	6±6.96	0.056 (NS)*
	LD	12	11±8.86		12	8±3.56		12	12±8.92	
	HD	12	14±5.31		10	7±1.91		10	14±6.21	
Daily feed intake (per rat, g)	Control	12	23±2.85	0.005 (HS)**	12	25±2.43	0.024 (S)**	12	22±4.70	0.004 (HS)**
	LD	12	20±3.82		12	23±3.14		12	20±4.79	
	HD	12	23±4.78		10	29±2.35		12	23±6.00	
Daily water intake (per rat, mL)	Control	12	32±5.57	0.000 (HS)**	12	32±4.54	0.029 (S)**	12	31±6.30	0.000 (HS)*
	LD	12	22±4.99		12	25±3.78		12	22±5.23	
	HD	12	24±3.97		10	28±3.43		12	25±4.37	
FER	Control	12	0.02±0.19	0.741 (NS)**	12	0.13±0.18	0.834 (NS)**	12	−0.32±2.01	0.825 (NS)**
	LD	12	0.05±0.18		12	0.13±0.14		12	−0.05±0.68	
	HD	12	0.06±0.14		10	1.11±0.09		12	−0.07±0.75	

*One-way ANOVA test; **Kruskal-Wallis test. FER: Feed efficiency ratio, LD: Low dose, HD: High dose, NS: Not significant, S: Significant, HS: High significant, SD: Standard deviation

Table 2: *Post hoc* analysis for the significantly different parameters in Table 1

Parameter	Group 1 (G1)	Group 2 (G2)	Initial period		Final period		Complete period	
			Mean difference (G1-G2)	*P*	Mean difference (G1-G2)	*P*	Mean difference (G1-G2)	*P*
Daily body weight (per rat, g)	Control	LD	−4	0.105 (NS)*	−13	0.011 (S)**	-6	0.016 (S)*
		HD	−19	0.000 (HS)*	−29	0.002 (HS)**	-22	0.000 (HS)*
	LD	HD	−15	0.000 (HS)*	−16	0.011 (S)**	-16	0.000 (HS)*
Total body weight gain (g)	Control	LD	−10	0.139 (NS)*	-	-	-15	0.053 (NS)*
		HD	−18	0.008 (HS)*			-20	0.013 (S)*
	LD	HD	−8	0.181 (NS)*			-5	0.475 (NS)*
Daily feed intake (per rat, g)	Control	LD	3	0.002 (HS)**	2	0.467 (NS)**	2	0.003 (HS)**
		HD	0	0.833 (NS)**	−4	0.024 (S)**	-1	0.498 (NS)**
	LD	HD	−3	0.017 (S)**	−6	0.029 (S)**	-3	0.006 (HS)**
Daily water intake (per rat, mL)	Control	LD	10	0.000 (HS)**	7	0.030 (S)**	9	0.000 (HS)*
		HD	8	0.000 (HS)**	4	0.195 (NS)**	6	0.000 (HS)*
	LD	HD	−2	0.195 (NS)**	−3	0.063 (NS)**	-3	0.062 (NS)*

**t*-test, **Mann-Whitney test. LD: Low dose, HD: High dose, NS: Not significant, S: Significant, HS: High significant

significantly lower than the respective control and HD groups, and the HD group for the final period was significantly higher than both the respective control and LD groups. The mean daily water intakes for the two doses for both the initial and complete periods and the LD group for the final period were all significantly lower compared to the respective controls.

Differential complete blood counts and serum IgM concentrations

There were no significant differences [Table 3] between the groups for the mean WBC and RBC counts for the first blood samples, and for the RBC and lymphocytes counts, and hemoglobin concentrations for the second blood samples. For the first blood samples [Table 4], the mean hemoglobin and IgM concentrations for the LD group were significantly lower than the respective concentrations for the control and HD groups. For the second blood samples [Table 4], the mean WBC, monocytes, and eosinophils counts for the LD and HD groups were all significantly higher compared to the mean counts for the respective controls. In addition, the mean neutrophil counts were significantly higher for the HD group compared to the control. The mean neutrophils percent for the HD group [Table 4] was significantly higher than for the LD group, and the mean monocytes and eosinophils percents for the LD and HD groups were both significantly higher compared to the respective controls. The mean lymphocytes percent for the HD group [Table 4] was significantly lower compared to both the control and LD groups.

Table 5 shows the differences between the first and second blood samples for the mean WBC and RBC counts, and hemoglobin concentrations. The only significant differences were significantly decreased mean RBC counts for the second blood samples for the control and HD groups compared to the respective first blood samples.

Discussion

Published studies using ground LSS or extracts on healthy laboratory animals are very few; thus, we were not able to compare all of our results with those of others. In addition, previous studies used different preparations and amounts of LSS than the ones used here. There are no previous studies on healthy subjects that compared any of the parameters used in this study between different experimental periods and there were no other studies that determined the amount of consumed water, IgM levels, and counts of WBC types in healthy subjects taking LSS in any form. Finally, this study is the first to use 15% LSS mixed with the diet for the studied parameters.

The present results show that both the daily body weight and total body weight gain were higher for rats consuming LSS. This is more pronounced for the HD group and for the longer experimental period. In agreement with these results, previous studies found higher mean daily body weights in mice[10] that consumed an aqueous LSS extract for 21 days and higher mean weekly body weights in rats[18] that consumed ground LSS seeds for 3 and 5 weeks compared to the controls. On the other hand, in disagreement with the current results, rats consuming ground LSS for 6 weeks[19] showed lower mean body weights while in mice[10] of the above study there was no significant difference in the mean total body weight gains compared to the controls. Other studies found no significant difference in the mean body weight gains in rats[8] that consumed LSS powder for 14 weeks, higher mean daily body weight gains in mice[10] and in chicks[17] that consumed different amounts of ground LSS mixed with the feed for 42 days, and lower mean body weight gains[14] in rats that consumed ground LSS for 6 weeks, each compared to its respective control.

Significantly lower daily feed intakes were found for the LD group for the initial and complete experimental periods, and a significantly higher intake for the HD group was found for the final period, compared to the respective controls. The HD rats had significantly higher feed intakes compared to the LD rats for all periods. The mean daily water intakes, compared to the control, were significantly lower for both the LD and HD rats for all experimental periods, with the exception of a no

significant difference for the HD group for the final period. The mean daily FER for the groups were not significantly different between the experimental periods.

These results of the feed intake for the LD group agree with the findings of a previous research study[14] that found a significantly lower feed intake in rats consuming LSS compared to controls. In addition, the result for the HD group for the final period agrees with the previous finding of a significantly higher feed intake for chicks[17] consuming LSS, compared to controls. Additionally, the study by Datta *et al*.[8] agrees with the unchanged feed intake for the HD group for the initial and complete periods, compared to the control, while it disagrees with the lower feed intake for the LD group. The unchanged FER results for the LD and HD groups, compared to the control, disagree with the significantly lower FER found by Adam.[14] There were no studies that determined the amount of consumed water in healthy subjects taking LSS in any form.

The mean IgM concentration for the LD group was significantly lower compared to the mean concentrations for the HD and control groups, but the HD group concentration was not significantly different from the controls. A previous study[17] found no difference in the total immunoglobulin concentration between chicks consuming ground LSS and controls in agreement with the IgM result for the HD group but not for the LD group. No other published studies determined levels of antibodies in healthy subjects consuming any form of LSS and none determined IgM levels.

For the HD group, the mean neutrophils count was significantly higher than the count for the control, while the neutrophils percent was significantly higher than the percent for the LD group. The mean lymphocytes percent for the HD group was significantly lower than the percents for both the control and LD groups. Both the mean monocytes and eosinophils counts and percents for the LSS groups were all significantly higher compared to the control. Only three other studies determined percents for the types of WBC in healthy subjects that consumed LSS, while none determined the counts. In agreement with the current findings, researchers[16] found significantly lower lymphocytes percent in rats that consumed LSS compared to the control. In disagreement with the current results, researchers found that rats that consumed ground LSS had significantly higher neutrophils[16] and lymphocytes percents[14] and significantly lower neutrophils,[14] monocytes, and eosinophils percents[16] compared to the control. Furthermore, in disagreement with the present findings, the study of Datta *et al*.[8] found no significant differences for neutrophils, lymphocytes, monocytes, and eosinophils percents in rats fed ground LSS.

Studies on mice administered LSS extract[10] and rats that received ground LSS[8] found no significant differences in the RBC counts and hemoglobin concentrations compared to controls. These findings agree with the current findings for mean RBC counts for the first and second blood samples, and mean hemoglobin concentration for the second blood samples, while they contradict the significantly lower mean hemoglobin concentration for the first blood sample. On the other hand, in contrast to the current findings, a study[17] found higher RBC counts and hemoglobin concentrations in chicks fed LSS.

Table 3: Statistical analysis for the differential complete blood counts and IgM concentrations for the first and second blood samples

Parameter	Group	*n*	Mean±SD	*P*
First blood sample				
WBC* ($\times10^3$ cell/μL)	Control	10	8.75±3.05	0.285 (NS)
	LD	11	9.25±2.37	
	HD	10	10.66±2.80	
RBC* ($\times10^6$ cell/μL)	Control	11	8.45±1.74	0.150 (NS)
	LD	11	9.95±2.26	
	HD	10	8.72±1.44	
Hemoglobin** (g/dl)	Control	11	15.439±0.827	0.002 (HS)
	LD	11	13.318±1.235	
	HD	10	14.720±1.147	
IgM** (ng/ml)	Control	12	636.339±498.022	0.005 (HS)
	LD	12	248.602±65.679	
	HD	12	437.820±281.320	
Second blood sample				
WBC** ($\times10^3$ cell/μL)	Control	12	7.51±2.31	0.040 (S)
	LD	12	10.03±2.84	
	HD	10	9.85±2.71	
Neutrophils* ($\times10^3$ cell/μL)	Control	10	2.29±0.74	0.038 (S)
	LD	12	2.67±0.77	
	HD	10	3.35±1.12	
Neutrophils* (%)	Control	10	29±6.76	0.028 (S)
	LD	12	27±4.54	
	HD	10	34±6.59	
Lymphocytes** ($\times10^3$ cell/μL)	Control	10	4.93±1.54	0.382 (NS)
	LD	12	5.99±1.62	
	HD	10	5.22±1.82	
Lymphocytes* (%)	Control	10	62±7.85	0.010 (S)
	LD	12	60±5.24	
	HD	10	53±7.93	
Monocytes** ($\times10^3$ cell/μL)	Control	10	0.51±0.26	0.011 (S)
	LD	12	0.89±0.35	
	HD	10	0.86±0.29	
Monocytes** (%)	Control	10	6±1.87	0.011 (S)
	LD	12	9±1.72	
	HD	10	9±2.38	
Eosinophils** ($\times10^3$ cell/μL)	Control	10	0.18±0.19	0.009 (HS)
	LD	12	0.49±0.37	
	HD	10	0.42±0.13	
Eosinophils* (%)	Control	10	2±2.10	0.023 (S)
	LD	12	5±2.35	
	HD	10	4±1.35	
RBC*** ($\times10^6$ cell/μL)	Control	7	6.76±1.05	0.684 (NS)
	HD	7	6.98±0.87	
Hemoglobin** (g/dl)	Control	12	14.738±1.390	0.230 (NS)
	LD	12	13.622±1.494	
	HD	10	14.399±0.796	

*One-way ANOVA test; **Kruskal-Wallis test. LD: Low dose, HD: High dose, NS: Not significant, S: Significant, HS: High significant, RBC: Red blood cell, WBC: White blood cell, SD: Standard deviation

Table 4: *Post hoc* analysis for the significantly different parameters in Table 3

Parameter	Group 1 (G1)	Group 2 (G2)	Mean difference (G1-G2)	*P*
First blood sample				
Hemoglobin* (g/dl)	Control	LD	2.121	0.001 (HS)
		HD	0.719	0.098 (NS)
	LD	HD	−1.402	0.024 (S)
IgM* (ng/ml)	Control	LD	387.737	0.002 (HS)
		HD	198.519	0.260 (NS)
	LD	HD	−189.218	0.030 (S)
Second blood sample				
WBC* ($\times 10^3$ cell/µL)	Control	LD	−2.53	0.030 (S)
		HD	−2.34	0.044 (S)
	LD	HD	0.21	0.468 (NS)
Neutrophils** ($\times 10^3$ cell/µL)	Control	LD	−0.38	0.325 (NS)
		HD	−1.06	0.012 (S)
	LD	HD	−0.68	0.084 (NS)
Neutrophils** (%)	Control	LD	2	0.378 (NS)
		HD	−5	0.076 (NS)
	LD	HD	−7	0.009 (HS)
Lymphocytes** (%)	Control	LD	2	0.430 (NS)
		HD	9	0.004 (HS)
	LD	HD	7	0.020 (S)
Monocytes* ($\times 10^3$ cell/µL)	Control	LD	−0.38	0.009 (HS)
		HD	−0.35	0.014 (S)
	LD	HD	0.03	0.621 (NS)
Monocytes* (%)	Control	LD	−3	0.008 (HS)
		HD	−3	0.018 (S)
	LD	HD	0	0.449 (NS)
Eosinophils* ($\times 10^3$ cell/µL)	Control	LD	−0.31	0.006 (HS)
		HD	−0.24	0.013 (S)
	LD	HD	0.07	0.869 (NS)
Eosinophils** (%)	Control	LD	−3	0.012 (S)
		HD	−2	0.021 (S)
	LD	HD	1	0.908 (NS)

*Mann-Whitney test, ***t*-test. LD: Low dose, HD: High dose, NS: Not significant, S: Significant, HS: High significant, WBC: White blood cell

The findings of no significant differences for the WBC counts for the LD and HD groups of the first blood samples agree with the findings on rats by Datta *et al.*[8] As for the second blood samples, compared to the control, the significantly higher mean WBC counts for the LSS groups for the second blood samples agree with the findings of other research studies on LSS in rats[16] and mice,[10] while they contradict the study[17] in chicks that found a lower WBC count. Only one study[14] determined the RBC and WBC counts, percent of types of WBC, and hemoglobin concentrations in rats administered LSS at two different experimental periods (3 and 6 weeks). In this study,[14] the WBC and RBC counts, and hemoglobin concentrations for the LSS rats were not significantly different from the control after 3 weeks, which agrees with the current findings for mean WBC and RBC counts for the first blood sample and for mean hemoglobin concentration for the second samples. In addition, the researchers found[14] that after administrating LSS for 6 weeks, WBC and RBC counts, and hemoglobin concentrations were all significantly lower in the LSS rats, which agrees with the current results for mean hemoglobin concentration for the first blood samples and disagrees with mean WBC and RBC counts for both blood samples and mean hemoglobin concentration for the second blood samples only.

Comparing WBC and RBC counts, and hemoglobin concentrations for the first and second blood samples, only RBC counts showed a significant difference. The HD diet resulted in a significant decrease in the RBC counts for the second samples compared to the first samples. There were no other studies on LSS that compared CBCs at different times during the experimental period.

Conclusions

LSS mixed with the diet lead to significantly higher mean body weights and total body weight gains and significantly

Table 5: Statistical analysis for the differences between the first and second blood samples for the mean white blood cell and red blood cell counts, and hemoglobin concentrations

Parameter	Group	Sample	*n*	Mean±SD	*P*
WBC* ($\times 10^3$ cell/μL)	Control	First	10	8.75±3.05	0.524 (NS)
		Second	10	7.88±2.30	
	LD	First	11	9.25±2.37	0.893 (NS)
		Second	11	9.38±1.83	
	HD	First	10	10.66±2.80	0.470 (NS)
		Second	10	9.85±2.71	
RBC* ($\times 10^6$ cell/μL)	Control	First	6	8.58±1.91	0.010 (S)
		Second	6	6.52±0.92	
	HD	First	7	9.02±1.60	0.037 (S)
		Second	7	6.98±0.87	
Hemoglobin** (g/dl)	Control	First	11	15.439±0.827	0.155 (NS)
		Second	11	14.673±1.439	
	LD*	First	11	13.318±1.235	0.473 (NS)
		Second	11	13.536±1.535	
	HD	First	10	14.720±1.147	0.678 (NS)
		Second	10	14.399±0.796	

*Paired *t*-test, **Wilcoxon test. LD: Low dose, HD: High dose, NS: Not significant, S: Significant, HS: High significant, RBC: Red blood cell, WBC: White blood cell, SD: Standard deviation

reduced feed and water consumptions compared to the control. LSS seem to affect humoral acquired immunity through significantly lower IgM concentration and lymphocytes percent, and affect innate immunity through significantly higher counts/percents of WBC, neutrophils, monocytes, and eosinophils. In addition, compared to the respective controls, mean WBC counts were significantly higher at the end of the experimental period but not for the shorter experimental period, while mean hemoglobin concentrations were lower for the first blood samples but not for the second samples. Therefore, the effects of LSS on WBC counts and hemoglobin concentrations depend on the duration of LSS ingestion.

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Conflicts of interest

There are no conflicts of interest.

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