Studying the potential healthy benefits of thyme (*Thymus vulgaris L.*) fed hyperlipidemic animal models targeting gut microbiota activities and compositions

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Abstract: Thyme (*Thymus vulgaris L.*), in arabic *zatar/satar*, used for long history as a medicinal healer and protector by improving and supporting the immune and respiratory systems additionally to help the digestive, nervous and other body systems. Our current study aimed to measure the influence of thyme -enriched diet on gut microbiota activities and compositions in correlation to hyperlipidemic condition; its implications for cholesterol management between animal models. Thyme has been added as fresh (5 and 10%) and dried (2 and 5%) to hyperlipidemic rats with negative and positive control groups for four weeks. Blood and feacal samples collected at the end of the experimental, then serum and colonic microbiota profiles (*Bifidobacteria*, *Clostridium histolyticum*, and *Lactobacillus)* were estimated. Data demonstrated significant ($p \leq 0.05$) health activities after feeding both fresh thyme concentrations (5 and 10%) comparing to the dried samples (2 and 5%). It has been shown that levels of cholesterol and HDL were declined significantly ($P \leq 0.05$) comparing to control (+) group. Also, gut microbiota compositions mainly *Bifidobacteria*, and *Lactobacillus* were higher after fresh samples consumption in contrast to the *Clostridium* levels that were decreased. Furthermore, short chain fatty acids (SCFAs; acetate, propionate and butyrate) had an increase with especial refer to colonic fuel; butyrate. To conclude up, feeding thyme (*zatar*) showed prospective effects on colonic microbiota composition in order to improve the quality of gut health which in turn could possibly serve as a novel therapeutic tool for hyperlipidemic patients. However, we recommend paying attention in the future to carry out more and more research in the area of medical plants and colonic bacterial interactions with extending its applications in human diets, industrial and medical fields.

Key words: Zatar/satar, hyperlipidemic , serum lipid profile, short chain fatty acids, *Bifidobacteria*, *Lactobacillus.*

Introduction

Two decades ago, gut microbiota was commonly shown increasing evidence in many experimental studies and dietary interaction wit many diseases. Colonic microbiota concentrations reaches up to 10^{14} total bacteria per gram faeces (Inna *et al.*, 2010; Laparra and Sanz, 2009). Such composition is dynamic and susceptible to any dietary changes that could affect the nutritional and health status of the host (Ley *et al.*, 2008). The metabolic end products of fermentation process by colonic microbioma include short chain fatty acids (SCFAs) that are produced and absorbed within the colon (Laparra and Sanz, 2009). Furthermore, SCFAs have a vital role in many biological processes such as allowing the absorption from the gut of key minerals (e.g. calcium, iron and magnesium) in addition to promote host health by, for instance, reducing risk factors associated with bowel cancer (Wong *et al.*, 2006). SCFAs levels; mainly acetate, propionate, butyrate has been found to be in a relative order consistent within human faecal samples, as follows: acetate > propionate ≥

butyrate and they are energy sources for host cells mainly acetate (muscle), propionate (liver) and butyrate (the colonic epithelium) (Cummings, 1997). The SCFAs levels is depending on the growth of colonic microbiota fermented/supplied substrate in addition to the inocula and its preparation which in total means all the culture conditions (Edwards *et al.*, 1996; Mortensen *et al.*, 1991; Mortensen *et al.*, 1992; Savage, 1986). So the amount of SFCA produced is dependent on the diet (Topping and Clifton, 2001). For instance, fermentation of retrograded maize starch increases the short chain fatty acids (Zhu and Zhao, 2013). Interestingly and most important, SCFAs have been induced proliferation of beneficial microbes such as *Bifidobacterium spp* and *Lactobacillus spp*. especially after fermentation of prebiotics in the gastrointestinal tract. So we are establishing an innovative study to evaluate the colonic microbiota composition and activities between hyperlipidimic animal models fed different sources and concentrations of thyme (*Zatar*).

Thyme is of the genus Thymus belongs to the mint family (*Lamiaceae*) which is the most common variety is *Thymus vulgaris*. Thyme is worldwide one of the most popular herbs that could be used either fresh or dried. It has been used for long time as a medicinal healer and protector especially with poisoning prevention. It was also used as anti-spoiled herbs with many foods e.g. meat (Sienkiewicz *et al.*, 20110). Thyme used as an excellent source for improving the human health through many systems such as immune, respiratory, digestive, nervous and other body systems in order to stay healthy. For instance, thyme has been used for lowering blood pressure and cholesterol levels by its antihypertensive activity. Its extract is also shown significantly reduction of heart rate with patients suffering from high blood pressure additionally to decreasing cholesterol, triglyceride and LDL levels (Alamgeer *et al.*, 2014). high fat levels in the blood e.g. cholesterol (CHO), triglycerides (TG), lipoproteins (VLDL and LDL) are associated factors for hyperlipidemia that is widely associated with many diseases such as cardiovascular disease (CVD) and atherosclerosis levels (Aslan *et al.*, 2010). According to our knowledge, previous researchers were not examined the colonic microbiota interaction and any of cholesterol-lowering therapy. Therefore, taking such relative data into considerations, support our study aim that is to measure the influence of thyme-enriched diet on gut microbiota activities and compositions in correlation to hyperlipidemic condition or its implications for cholesterol management, lifestyle changes in considerations.

Materials and methods

Materials

Fresh and dried thyme (*Zatar*) have been obtained from the Egyptian National Institute, Cairo, Egypt. All used chemicals in grade analysif and kits were obtained from Sigma-Aldrish corp., Cairo, Egypt.

Experimental design

In the present study, adult male albino rats, weighting 160± 10 acquired from the animal House of The National Research Center, Dokki, Egypt. All animal were accommodated in well-aerated cages then fed standard diet for adaptation period and consumed diets were prepared as described previously by the American Institute of Nutrition (AIN, 1993) recommendation for rodent growth. Fresh (5 and 10%) and dried (2 and 5%) thyme were fed to hyperlipidemic rats. Hyperlipidemic induced by feeding the rats' high fat diet for a month. After that the experimental carried out for another month time; the rats were divided into four groups in addition to two used groups as positive and negative as follows**:**

- Group 1 (**G1**): (Control negative), rats fed basal diet.
- Group 2 (**G2**): (Control positive), hyperlipidemic rats fed on basal diet.
- Group 3 (**G3**): Hyperlipidemic rats fed basal diet + 5 % fresh Thyme.
- Group 4 (**G4**): Hyperlipidemic rats fed basal diet + 10 % fresh Thyme.
- Group 5 (**G5**): Hyperlipidemic rats fed basal diet + 2 % dried Thyme.
- Group6 (**G6**): Hyperlipidemic rats fed basal diet + 5 % dried Thyme.

Sample collection and preparation

All collected blood samples were processed in order to determine serum glucose, lipid profile (cholesterols, triglycerides ...). Faecal and blood samples were collected during running the experimental (0, 2 and 4 weeks of feeding thyme). Faecal homogenates are prepared as described previously (Khalil *et al.*, 2013). In brief, faecal samples were dried and weighed prior to phosphate-buffered saline (PBS, pH 7.2) additions for obtaining a final concentration of 20mg/mL and finally the mix was homogenized, centrifuged, and supernatants were frozen at −20°C for colonic microbiota evaluations. Additionally, some homogenized feacal samples have been used for SCFAs determinations as described previously by our team (Khalil *et al.*, 2013).

Determination of SCFA

The levels of SCFAs was performed as described before (Khalil *et al.*, 2013) using gas-liquid chromatography (GC) using an HP 5890 series II GC system (Hewlett Packard, Palo Alto, California) equipped with a capillary fused silica-packed column (Permabond FFAP J&W Scientific). Elution times were recorded, and the data were then analyzed using principal component analysis.

Biochemical parameters

Biological parameters were evaluated by collected blood sampling for determination of serum lipid profile. Total serum cholesterol; evaluated according to the method described by Thomas (1992). Triglycerides (TG) evaluated in the serum as described by Fossati and Prencipe, (1982). Very low Density lipoprotein (VLDL); evaluated according to Lee and Nieman (1996). Low density lipoprotein (LDL); calculated according to Lee and Nieman (1996). High density lipoprotein (HDL) was evaluated according to the method described by Allain *et al.*, (1974).

Statistical analysis

All Collected data were performed using one-way analysis of variance (ANOVA) and were revealed as mean ± standard deviation (SD). Differences among means in triplicates were tested subsequently using post-hoc test, Duncan's multiple range. Data were considered significant statistically differences at P≤ 0.05.

Results and discussion

The current study established to measure the actual effects of different fresh and dried thyme (*zatar*) concentrations between hyperlipidemic animal models; rats' health. Table 1 show such effects on serum glucose levels and data are in mean of six used rats in each group. It can be seen (Table 1) that the heist glucose levels were with G2; the control positive group which is about 250mg/dl and that was significantly difference with all the other groups ($P \leq 0.05$) suggesting the health benefits of thyme (*zatar*) fed all of the groups; positive significantly effects on serum glucose reduced levels ($P \leq 0.05$). The biggest reduction significantly ($P \leq 0.05$) was seen with the rats group**; G6** that consumed diets supplemented with**;** 5 % dried thyme (more than 50% reductions of positive control group).

Table (1): Effect of thyme supplementations on blood glucose levels between hyperlipidemic rats

Groups	Glucose level (mg.dl^{-1})	Relative % change of control (+) group
G1; Control Negative	98.60±3.27^{f}	60.30
G2; Control Positive	248.40±3.62^{a}	----
G3; 5% fresh Thyme	144.93±2.25^{b}	41.65
G4; 10% fresh Thyme	133.77±2.46^{c}	46.15
G5; 2% dried Thyme	127.10±4.25^{d}	48.83
G6; 5% dried Thyme	116.80±2.95^{e}	52.98

Data represent mean ± SD. Values in the same column were not sharing superscript letters are significantly different at $p \leq 0.05$.

The results of different parameters measured in table 2 show the influence of thyme supplementations on lipid profile levels between hyperlipidemic rats. Total cholesterol, Triglycerides, HDL, LDL and VLDL were measured. Firstly, total cholesterol data in Table (2) indicated that levels were declined within all the groups after thyme additions with all the supplied concentrations. However, the most effective concentration significantly ($P \leq 0.05$) was seen with G6; after feeding rats 5% dried thyme with values of about 100mg/dl. Also, both of G4 and G5 whom consumed 10% of fresh thyme and 2% dried thyme, respectively are giving about the same declined levels of total cholesterol comparing to the control positive group; G2. Secondly, analysis of triglycerides levels again were decreased with all rats consumed thyme supplementations. However, the highest effect significantly ($P \leq 0.05$) was seen between all the groups with G6 that ate 5% dried thyme; about the same as the control negative group (about 68 mg/dl each group). Additionally, HDL levels were also reduced with all the groups fed thyme with different levels of percentages; either fresh or dried thyme. However, the huge impact was seen with G6 rats; consumed 5% dried thyme (to approximately 30 mg/dl). Also, both G4 (10% fresh thyme) and G5 (2% dried Thyme) give about the same reduced levels; approximately 53 mg/dl).

On the other side, LDL levels were at the lowest levels with G2; Control Positive group (34mg/dl) and in addition it was increased with all the groups consumed thyme in comparison to the control positive group. The maximum increase was seen with G6; dried Thyme 5% by about 24 mg/dl of positive control group. Furthermore, VLDL data had shown reduction with all supplemented thyme groups and again such reduction is about the same as the control negative group (about 13 mg/dl each group). Overall, the highest effective concentrations were seen after addition of 10%fresh followed by 5% dried thyme in comparison with the control samples. All collected data regarding the thyme addition's effect were seen early with a study that was using Thymus extract. Data of this study are in accordance with that obtained by many authors (Alamgeer *et al.*, 2014) as such data with the others confirmed that serum cholesterol, triglycerides and HDL levels reduced while an increase in LDL level was observed significant ($P \leq 0.05$). Also, over the past decades different studies have evaluated the antibacterial activities of essential oils from Thymus vulgaris and demonstrated the highest antimicrobial activities with its essential oils (Sienkiewicz *et al.*, 2011 and Magi *et al.*, 2015).

Table (2): Effect of thyme supplementations on lipid profile levels between hyperlipidemic rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
G1; Control Negative	94.37 ± 1.32^{e}	68.50 ± 1.27^{d}	15.83 ± 4.50^{d}	64.83 ± 5.75^{a}	13.7 ± 0.25^{d}
G2; Control Positive	253.67 ± 1.41^{a}	117.73 ± 2.9^{a}	195.4 ± 3.53^{a}	34.7 ± 2.84^{d}	23.55 ± 0.58^{a}
G3; 5% fresh Thyme	197.13 ± 4.62^{b}	83.97 ± 1.30^{b}	144.37 ± 3.28^{b}	35.96 ± 3.49^{d}	16.79 ± 0.26^{b}
G4; 10% fresh Thyme	114.13 ± 1.42^{c}	74.73 ± 3.06^{c}	52.5 ± 1.61^{c}	46.66 ± 0.60^{c}	14.95 ± 0.61^{c}
G5; 2% dried Thyme	114.57 ± 2.75^{c}	73.63 ± 1.13^{c}	$53..27\pm4.06^{c}$	46.56 ± 1.45^{c}	14.73 ± 0.22^{c}
G6; 5% dried Thyme	101.67 ± 1.45^{d}	68.96 ± 0.49^{d}	29.8 ± 3.46	58.03 ± 2.13^{b}	13.79 ± 0.09^{d}

Data represent mean ± SD. Values in the same column were not sharing superscript letters are significantly different at $p \leq 0.05$.

Data in Table (3) show the effects of dried thyme supplementations on gut microbiota compositions between hyperlipidemic rats. Results of gut microbiota compositions after 4 weeks period affected by dried thyme (2 and 5%) have significant ($P \leq 0.05$) higher effects (5.98×10^{6} and 6.10×10^{6} bacteria/ml) than the fresh (5 and 10%) samples on probiotics colonic bacterial species with especial refer to *Bifidobacteria* (5.49×10^{5}, 5.69×10^{5} bacteria/ml). Data of probiotics species were grown statistically difference between the control samples (without any addition of thyme) and all the other supplemented diabetic groups. This affect was significantly

($P \leq 0.05$) started with the addition of low levels of fresh thyme; 5% between G3; and also such effect seen with increasing the amount of thyme gradually. Again the highest addition of dried thyme; 5% within G6 resulted in a difference of (0.74×10^6 bacteria/ml) for *Bifidobacteria* and 0.36×10^6 bacteria/ml) for *Lactobacillus* in comparison with the control negative sample. However and on the other side, the highest addition 5% dried or 10% fresh of thyme resulted in low levels of *Clostridium histolyticum group* statistically significant ($P \leq 0.05$) with gradually decreased in comparison with the control sample.

Figure (1) with the current study presenting SCFAs levels produced after feeding different fresh and dried thyme concentrations between hyperlipidemic animal models. Previous literature revealed that the overall production of SCFA formed are predictable to be indicative of the quantity of fermentation activity with the in vitro gut cultures (Topping and Clifton, 2001). So the highest amount of SCFAs release is the growth of colonic microbiota depending on the fermented/supplied substrate in addition to the inocula and its preparation which in total means all the culture conditions as it has been demonstrated previously (Edwards et al., 1996; Mortensen et al., 1991; Mortensen et al., 1992; Savage, 1986). SCFAs levels; mainly acetate, propionate, butyrate has been found to be in a relative order consistent within human faecal samples, as follows: acetate > propionate ≥ butyrate and such data are in agreements with previous studies (Khalil et al., 2013; Flwming et al., 1985; Hoverstad and Bjornklett, 1984).

There are notable differences in the production of butyrate and propionate levels produced after thyme additions especially at high concentrated fresh levels (10%) and both used dried levels (2 and 5%). The animal groups fed either10% fresh thyme or any of the dried used concentrations showed higher levels of butyrate productions comparing to the other groups and that were a bit close to the levels generated by the control healthy group; with the normal diet (Figures 1). This effect could be significant as butyrate production in hyperlipidemic correlated diseases has been found to promote disease recovery through stimulating proliferation of the gut mucosa and dietary provision that are fermented to SCFAs (Bamba *et al.*, 2002; Tuohy *et al.*, 2005). Furthermore, SCFAs as metabolic end products of colonic microbiota have been induced proliferation of beneficial microbes (mainly *Bifidobacterium spp* and *Lactobacillus spp*.) especially after fermentation of prebiotics in the gastrointestinal tract. Thus the current butyrate levels produced within all the animal groups is reflecting the growth levels of the colonic microbiota with especial respect to *Bifidobacterium spp* and *Lactobacillus spp* within G4, G5 and G6. Again, figure 1 demonstrated that acetate levels were relatively higher within the groups of small concentrated thyme supplementations in contrast to the lowest levels within the other groups especially and again within G4, G5 and G6 which means that acetate had shift to butyrate in response to thyme fermentation. Such data are considerable effective and worth of further study, however Belenguer *et al.*, (2006) demonstrated that there is a cross feeding network where butyrate is produced from the breakdown of acetate. It is meaning that an improvement in the quality of gut health occurred after thyme consumption in either fresh or dried form. From the viewpoint of thyme was found to have a prebiotic effects resulting in positive effects on both colonic bacteria and both SCFAs types and levels.

Table (3): Effect of dried sage supplementations on gut microbiota compositions between hyperlipidemic rats

Treatments	Time (week)	Bacterial counts (Log [10] cells /ml feacal slurry)		
		Bifidobacteria	*Clostridium histolyticum group*	*Lactobacillus*
G1; Control Negative	0	$6.08\times10^{6}\pm 0.08^{a}$	$5.40\times10^{5}\pm0.05^{g}$	$6.26\times10^{6}\pm0.02^{a}$
	2	$6.07\times10^{6}\pm0.08^{a}$	$5.41\times10^{5}\pm0.10^{g}$	$6.18\times10^{6}\pm0.02^{abc}$
	4	$6.11\times10^{6}\pm0.01^{a}$	$5.45\times10^{5}\pm0.04^{fg}$	$6.21\times10^{6}\pm0.01^{eb}$
G2; Control Positive	0	$5.34\times10^{5}\pm0.05^{e}$	$6.17\times10^{6}\pm0.05^{a}$	$5.91\times10^{5}\pm0.02^{de}$
	2	$5.36\times10^{5}\pm0.04^{e}$	$6.16\times10^{6}\pm0.05^{a}$	$5.91\times10^{5}\pm0.02^{de}$
	4	$5.37\times10^{5}\pm0.02^{e}$	$6.13\times10^{6}\pm0.04^{ab}$	$5.92\times10^{5}\pm0.02^{de}$
G3; 5% fresh Thyme	0	$5.34\times10^{5}\pm0.04^{e}$	$6.13\times10^{6}\pm0.05^{ab}$	$5.92\times10^{5}\pm0.03^{de}$
	2	$5.47\times10^{5}\pm0.05^{d}$	$6.03\times10^{6}\pm0.02^{bc}$	$5.94\times10^{5}\pm0.05^{de}$
	4	$5.49\times10^{5}\pm0.06^{d}$	$5.97\times10^{5}\pm0.04^{c}$	$5.97\times10^{5}\pm0.04^{de}$
G4; 10% fresh Thyme	0	$5.34\times10^{5}\pm0.03^{e}$	$6.10\times10^{6}\pm0.10^{ab}$	$5.90\times10^{5}\pm0.02^{de}$
	2	$5.70\times10^{5}\pm0.02^{c}$	$5.77\times10^{5}\pm0.01^{d}$	$5.98\times10^{5}\pm0.08^{d}$
	4	$5.69\times10^{5}\pm0.02^{c}$	$5.55\times10^{5}\pm0.02^{ef}$	$6.12\times10^{6}\pm0.04^{bc}$
G5; 2% dried Thyme	0	$5.36\times10^{5}\pm0.02^{e}$	$6.11\times10^{6}\pm0.01^{ab}$	$5.91\times10^{5}\pm0.05^{de}$
	2	$5.97\times10^{5}\pm0.01^{b}$	$5.76\times10^{5}\pm0.03^{d}$	$6.16\times10^{6}\pm0.04^{bc}$
	4	$5.98\times10^{5}\pm0.05^{b}$	$5.52\times10^{5}\pm0.07^{ef}$	$6.17\times10^{6}\pm0.05^{bc}$
G6; 5% dried Thyme	0	$5.34\times10^{6}\pm0.04^{e}$	$6.12\times10^{6}\pm0.05^{ab}$	$5.89\times10^{5}\pm0.03^{e}$
	2	$6.08\times10^{6}\pm0.03^{a}$	$5.59\times10^{5}\pm0.04^{e}$	$6.11\times10^{6}\pm0.09^{c}$
	4	$6.10\times10^{6}\pm0.04^{a}$	$5.53\times10^{5}\pm0.08^{ef}$	$6.18\times10^{6}\pm0.03^{abc}$

Data prove mean ± SD. Values in the same column were not sharing superscript letters are significantly different at $p \leq 0.05$.

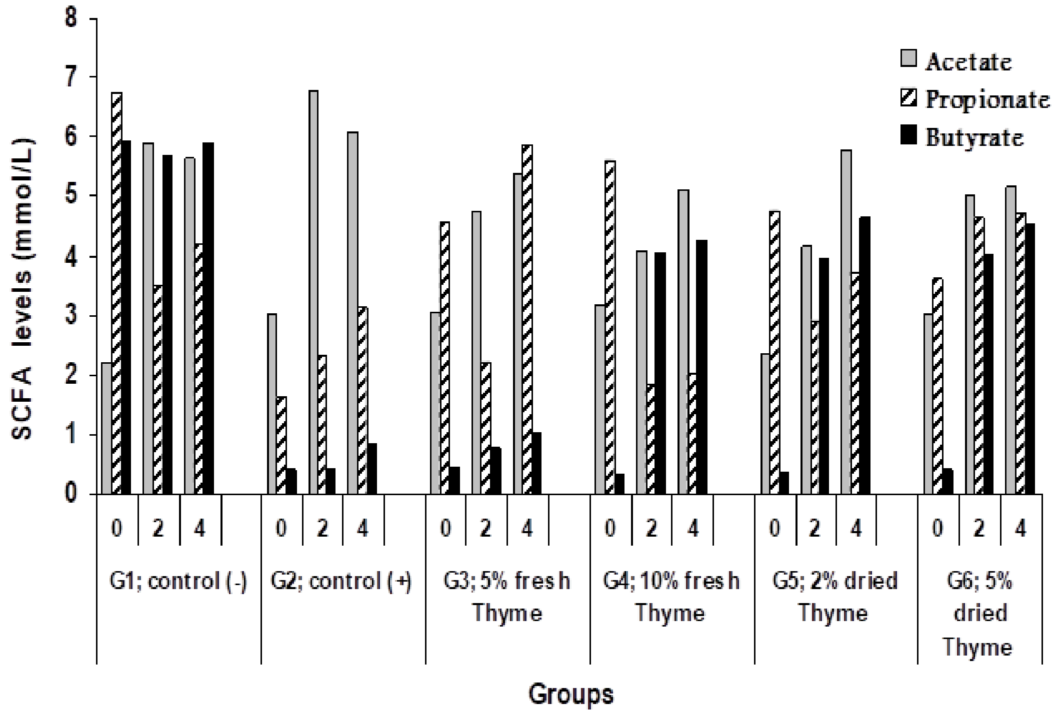

Figure (1): Effects of thyme supplementations on SCFAs levels between hyperlipidemic rats.

Conclusion

The results reveal that the addition of either fresh or dried thyme has significant (P≤0.05) improvements on the hyperlipidemic animal model's health. Additionally, colonic microbita composition and activities had potentially health benefits on the gut health by butyrate productions which in turn could possibly serve as a novel therapeutic tool for hyperlipidemic patients. Thus, we recommend paying attention in the future to carry out more and more research in the area of thyme and colonic bacterial interactions with extending its applications in human diets, industrial and medical applications which have healthy effects for the human being.

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دراسة الفوائد الصحية المحتملة للزعتر (*Thymus vulgaris*) على الحالة الصحية لحيوانات التجارب المصابة بارتفاع نسبة الدهون بالدم: تستهدف الدراسة أنشطة و نسب بكتريا القولون

نزيهة عبدا لرحمن إبراهيم خليل

قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي - جامعة المنوفية - شبين الكوم- مصر

الملخص

يستخدم الزعتر (*Thymus vulgaris*) لتاريخ طويل كمعالج وحامي طبي من خلال تحسين ودعم الجهاز المناعي والجهاز التنفسي بالإضافة إلى مساعدة الجهاز الهضمي والعصبي وغيره من أجهزة الجسم المختلفة فى أداء وظائفها بكفاءة عند الإصابة بالإعتلال. لذلك تهدف الدراسة الحالية الى إستكشاف تأثير التغذية بنبات الزعتر على الميكروبات المستوطنة في الأمعاء بين النماذج الحيوانية المصابة (فئران) بارتفاع نسبة الدهون بالدم. تمت إضافة الزعتر كغذاء طازج (5 ، 10٪) ومجفف (2 ، 5٪) إلى فئران لخفض نسبة الدهون مع مجموعات ضابطة سالبة و أخرى موجبة لمدة أربعة أسابيع. تم تقدير عينات الدم مع البراز التي تم تجميعها في نهاية التجربة، ثم الدهون (الكولسترول، الدهون الثلاثية...) مع البكتريا المجهرية المستوطنة بالقولون (البيفيدوبكتريا ، كلوستريديوم هيستوليتيكوم ، لاكتوباسيلس). أظهرت النتائج حدوث أنشطة صحية كبيرة بعد التغذية بتركيزات الزعتر الطازجة (5 ، 10 ٪) مقارنة مع العينات المجففة (2 ، 5 ٪). وقد تبين أن مستويات الكوليستيرول والـ HDL انخفضت بشكل كبير معنوي ($p \le 0.05$) مقارنة مع المجموعة الضابطة (+). ومرة أخرى ، كانت التركيبات المجهرية للأمعاء هي أساسًا البيفيدوبكتريا ، و اللاكتوباسيلس أعلى بعد استهلاك عينات جديدة على النقيض من مستويات كلوستريديوم التي انخفضت. أيضا الأحماض الدهنية قصيرة السلسلة (SCFAs : أسيتات، بروبيونات، بيوتيرات) كانت أكثر إنتاجا لحمض البيوتيريك الذي يعد مفيدا ويعمل على تحسين الحالة الصحة للأمعاء. والخلاصة، تفيد الدراسة أن التغذية بالزعتر لها تأثيرات على تكوين البكتريا المستوطنة بالأمعاء الدقيقة و التي لها دور في تحسين الحالة الصحية للفئران المصابة بارتفاع نسبة الدهون بالدم، كما انها من المهم التوسع بدراستها مستقبليا.

الكلمات المفتاحية : ارتفاع نسبة صورة دهون الدم، الدهون فى الدم، الأحماض الدهنية قصيرة السلسلة، البيفيدوبكتريا، لاكتوباسيلس