Evaluating The Nutritional and Immune-boosting Characteristics of Functional *Lepidium sativum* Fermented Milk in Male Albino Rats

M. A. E. Gomaa [1], Ahmed R. A. M. Awd [2], Khater A. Khater [2], Samir O. A. Shalaby [2]

1 Food Science Department, Faculty of Agriculture Saba Basha, Alexandria University, Alexandria, Egypt.
2 Dairy Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

DOI: 10.21608/JALEXU.2023.202224.1129

Article Information

Received: March 14th 2023

Revised: March 22nd 2023

Accepted: April 9th 2023

Published: June 30th 2023

ABSTRACT: The nutritional and immune-enhancing properties of functional *Lepidium sativum* fermented milk were studied in the male albino rat model. The effects of the product on daily weight gain (DWG), specific growth rate (SGR%), relative growth rate (RGR), feed conversion ratio (FCR), immunoglobulins (IgG and IgM), lymphocytes, monocytes, eosinophils, basophils, and neutrophils were studied in five experimental groups. Following a 21-day acclimation period, the experiment was conducted for 70 days. The results showed that all sensory properties of the product were comparable to those of the control and showed excellent acceptability with a rating of 90%. *Lepidium sativum* significantly affected the weight of the rats during the first week. Then, at the end of the experiment, a higher weight gain was observed in the treated groups. The most effective treatment to restore the weight of rats was 3%. SGR%, RGR, and FCR showed the same trend. IgM was significantly improved in all groups except the control (+) and the group treated with *Lepidium sativum* 1%. In the three groups treated with *Lepidium sativum* 1, 2 and 3% and *Bifidobacterium longum*, a gradual significant increase was observed depending on the dose. The highest value was observed in the case of 3% *Lepidium sativum* with 61.33±4.67. The number of lymphocytes, monocytes, eosinophils, and neutrophils indicated that *Lepidium sativum* combined with *Bifidobacterium longum* alleviated immunity dose-dependently. Administration of 3% *Lepidium sativum* was more effective than 1 and 2% for most parameters, indicating that 3% *Lepidium sativum* with probiotic fermentation enhanced immunological activity in male albino rats.

Keywords: *Lepidium Sativum; IgM; IgG; Lymphocytes; Fermented milk; Bifidobacterium longum; Growth performance*

INTRODUCTION

Functional foods are currently in vogue. The dairy market has great potential to be the main player in this trend with significant benefits and affordable prices. Consumption and production of functional products are increasing, especially in the dairy market. Among the most popular products are fermented milk and cheese, to which prebiotics and probiotics are added and which are rich in bioactive compounds or vitamins and minerals **(Brunner, 2005; Costa *et al.*, 2010; Valero-Cases *et al.*, 2020; Balthazar *et al.*, 2022)**. Dietary habits play a fundamental role in human health. This is why so many people are concerned about their health and well-being because the quality of life is a reflection of a healthy and balanced diet and physical activity **(Cooper, 2013; Asioli *et al.*, 2017)**.

The immune system of vertebrates consists of several defence mechanisms, from mucosal and epithelial cells which form the exterior barrier to the genetically programmed pathogen-recognition receptors on immune cells. The innate immune system is our initial line of defence against a pathogenic onslaught. It reacts quickly by employing receptors that can identify patterns on either viruses or the chemicals they emit. These pathogen-released chemicals, also known as alarmins, are recognized by attaching to immune cell receptors, which activate signalling pathways that kill the invasive bacteria **(Bianchi, 2007; Bishayee, 2009)**.

Hachimura *et al.* **(2018)** stated that the immune system is broadly divided into innate (nonspecific) and adaptive (specific) immunity. Specific immunity is associated with immunoglobulins (Ig), which are produced by B cells in response to antigenic attacks, such as pathogens and allergens. The Igs are further subdivided into the classes IgA, IgG, and IgE. All are produced by isotype switching upon activation. Serum immunoglobulin levels provide important information about the humoral immune status. Some humoral immunodeficiencies are defined by low immunoglobulin (Ig) levels

(Buckley, 1986). On the other hand, liver problems, chronic inflammatory diseases, hematologic disorders, infections, and malignancies are associated with elevated immunoglobulin levels (polyclonal gammopathy) **(Dispenzieri *et al.*, 2001)**.

To our knowledge, few studies are addressing the potential impact of functional foods derived from milk on boosting immunity in general and serum immunoglobulin levels. Besides, prebiotics may also regulate the host immune response by altering the gut microbiota. Besides, the indigestible oligosaccharide raffinose significantly suppressed IgE elevation in a food allergy and inhibited the Th2 response characteristic of the onset of food allergy **(Nagura *et al.*, 2002)**.

Garden cress (*Lepidium sativum*) is an edible annual herbaceous plant of the *Cruciferae* family. while the leaves are eaten in a salad, Garden cress seeds are utilized in a variety of medical uses: A immune system booster, for the treatment of dysentery, anemia, diarrhea, migraine, and diabetic **(Eddouks and Maghrani, 2008)**. In addition, it has antiasthmatic, antioxidant, anticarcinogenic, antimutagenic, hypolipidemic, antibacterial, antifungal, and anti-inflammatory activity **(Aburjai *et al.*, 2001)**. It is also good for healing bone fractures and has protective and curative effects against nephrotoxicity and fatty liver in albino rats. The therapeutic effect of garden cress is attributed to the presence of active therapeutic compounds and antioxidant phytochemical compounds **(Sakai *et al.*, 2014)** such as alkaloids, tannins, flavonoids, phenols, riboflavin, thiamine, niacin, α-tocopherols, β-carotenes, β-sitosterol, ascorbic acid, linolenic acid, oleic acid, palmitic acid, stearic acid, and amino acids (glutamine, cysteine, and glycine) **(Gaafar *et al.*, 2013; Jain *et al.*, 2016)**, and minerals (calcium, magnesium, iron and zinc). Therefore, they play an important role in many metabolic processes and the performance of important biological functions throughout the life cycle **(Gaafar *et al.*, 2013)**.

This study assesses the effects of functional dairy products enriched with garden cress at different concentrations (1, 2, and 3%) and a probiotic strain (*Bifidobacterium longum* ATCC15707) on the immune system linked to immunoglobulins (Ig) in albino rats.

2. MATERIALS AND METHODS

2.1. Materials

Seeds of *Lepidium Sativum* and fresh full-fat buffalo milk were purchased from the local market in Alexandria. The milk samples were collected and transported in an icebox under cooling to the laboratory where the experiments were performed within 60 min.

Cultures of lactic acid bacteria (LAB) (*Lactobacillus delbrueckii* subsp. *bulgaricus* STY9 and *Streptococcus thermophilus* STY1) were obtained from the Food Science Department, Faculty of Agriculture, Saba Basha, Alexandria University, Culture Collection (FABA). *Bifidobacterium longum* ATCC15707 was kindly provided by Dr. Nassra Dabour, Faculty of Agriculture Al-Shatby, Alexandria University, American Type Culture Collection (ATCC).

The basal diet for the feeding experiment was M.R.C. Diet 41 **(Bruce and Parkes, 1949) with modifications** as follows: Corn starch (67.6%), casein (11.9%), corn oil (10%), salt mixture (4%), vitamin mixture (1%), barn (5%), methionine (0.3%), and choline chloride (0.2%).

2.2. Chemical analysis of *Lepidium Sativum* and milk

The seeds of *Lepidium Sativum* were subjected to chemical analysis to determine moisture, protein, fat, ash, and crude fibre **(William, 2000).** Total carbohydrates were determined by difference.

The fat, soluble non-fat (SNF), protein, lactose, density, freezing point (calculated), and ash of the milk samples were determined using the Funke Gerber 3510 Laktostar milk content analyzer (Funke Gerber, Berlin, Germany) based on a thermo-optical procedure combination. Besides, the pH values of the milk were measured using a pH meter (Jenway 3505, England).

2.3. Conditions for the strains' cultivation

For selective enumeration of *Lactobacillus delbrueckii subsp. bulgaricus* STY9, pH-modified (4.58) MRS (deMann, Rogasa, and Sharpe) agar was used (Oxoid Ltd., Hampshire, England). M-17 agar (Oxoid Australia Ltd) was used for the enumeration of *Streptococcus thermophilus* STY1 (Dave & Shah, 1996). MRS-NNLP Agar (MRS-nalidixic acid, neomycine sulfate, lithium chloride and paromomycine sulfate) was prepared for the enumeration of *Bifidobacterium longum* ATCC15707 (Dave & Shah, 1996). All bacteria were incubated in anaerobic jars (AnaeroGen TM, Oxoid Ltd., Hampshire, England), except for *S. thermophilus*, which was incubated under aerobic conditions. Incubation temperatures and times for *L. delbrueckii* subsp. *Bulgaricus*, *S. thermophilus*, and *B. longum* were 37 °C (24 h), 45 °C (72 h), and 30 °C (72 h), respectively.

2.4. Fermented milk manufacture

The fermented milk was prepared by lactic acid fermentation of buffalo milk. The milk was heated at 80°C for 10 minutes. Probiotic

starter cultures (*Lactobacillus delbrueckii subsp. Bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium longum* ATCC15707) were added in a ratio of 1:1:1 to reach 10^8 CFU/mL in the final mixture at 42 ºC and mixed well. The inoculated milk was poured into cups (100 mL each) and incubated at 42 ºC for about 3 h to reach a pH of 4.5 - 4.6, followed by cooling at 4 °C. Three treatments with *Lepidium Sativum* seeds at different concentrations (1, 2, and 3% w/w) were prepared, in addition to the control.

2.5. Viscosity and pH

The viscosity of the fermented milk treatments was measured at 15 °C using a viscometer (D.P. SELECTA, S.A. ST-2020R, Korea) at a speed of 60 to 200 rpm with spindle R5; the temperature was automatically corrected. The viscosity of the fermented milk was expressed in mPa·s.

The pH values of the fermented milk samples were measured with a pH meter (Jenway 3505, England).

2.6. Diacetyl and acetaldehyde content

For the determination of the diacetyl, the method of Owades and Jakovac modified by Pack *et al.* **(1964)** was used. Approximately, 1g of sample was used to suit the modern equipment. Acetaldehyde was determined on a separate aliquot of the culture at the same time as diacetyl by a modified 3-methyl-2-benzothiazolone hydrazone procedure **(Lindsay and Day, 1965)**.

2.7. Sensory evaluation

Nine experienced panellists, consisting of students and staff of the Faculty of Agriculture, Saba Basha, Alexandria University, aged 20–61 years, participated in the evaluation of sensory attributes of fermented milk samples one day after production. Fermented milk samples (100 mL cups) were placed on white plates and randomly presented to panellists. Panellists were asked to evaluate the sensory attributes of the fermented milk (flavour, appearance, and textural properties) using a 5-point scale (1 being the worst score and 5 being the best score).

First, the smell was assessed by removing the lid of the cup and rating the intensity of the volatile smell substances. Second, appearance was assessed by visual observation and textural properties by breaking the fermented milk gel and agitating the product. Finally, the taste of the product was evaluated by swallowing 10 g (a teaspoon portion) of the sample. Overall acceptance was rated at the end of the sensory evaluation of each sample **(Soukoulis *et al.*, 2007)**.

2.8. Feeding experimental design

Thirty adult male healthy albino rats weighing 205 ± 5 g were housed in wire cages under hygienic conditions and a good ventilation system in the animal house of the Faculty of Medicine, Alexandria University. The rats were fed on a basal diet for twenty-one consecutive days adaptation period as recommended by the veterinarian. The rats were then divided into five groups of 5 rats each, except for the negative control group which contained ten rats. The weight of the rats was almost the same in all groups and was monitored throughout the experiment (70 days). The basal diet was mixed with fermented milk prepared with *Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus*; the experimental groups were divided as follows:

Group (1): Negative control group, in which the rats were fed only a basal diet throughout the experimental period.

Group (2): Positive control group was fed with a basal diet containing probiotic bacteria (*Bifidobacterium longum*; 10^8 CFU/g).

Groups (3), (4), and (5): Rats were fed basal diets mixed with *Lepidium Sativum* at concentrations of 1, 2, and 3% (1:1 by weight), respectively, in addition to *Bifidobacterium longum* (10^8 CFU/g) in all groups.

2.9. Growth performance parameters

The weight of the rats was recorded daily during the experiments. At the end of the experiment, final body weight gain (FWG; g), daily weight gain (DWG; g/D), feed conversion ratio (FCR; g), specific growth rate (SGR %/D), and relative growth rate (RGR %) were calculated according to the following equations (1), (2), (3), (4), and (5), respectively:

$$\mathbf{FWG\ (g) = WF - W0} \quad \mathbf{(1)}$$

Where: (WF) is the final weight and (W0) is the initial body weight

$$\mathbf{DWG\ (g/D) = WF - W0/n.} \quad \mathbf{(2)}$$

Where: (WF) is the final weight, (W0) is the initial body weight, and (n) is the period duration.

$$\mathbf{FCR\ (g) = dry\ matter\ intake\ (g)/body\ weight\ gain\ (g)} \quad \mathbf{(3)}$$

$$\mathbf{SGR\ (\%/\ day) = 100 \times (ln\ WF - ln\ W0)/\ days.} \quad \mathbf{(4)}$$

Where: (ln WF) is the natural logarithm of the final weight and (ln W0) is the natural logarithm of the initial body weight

RGR (%) = 100× (average final weight/average initial weight) (5)

2.10. Quantification of IgG, IgM, and lymphocyte concentrations

Plasma samples from rats were obtained from blood samples and centrifuged. The concentrations of IgG and IgM were measured by direct ELISA compared with rat IgG and rat IgM standards. The IgG-ELISA was developed by Taday and Eigenbrodt **(1998)**.

The evaluated hematology device Cell-Dyn® 3500 CS (Abbott, Wiesbaden-Delkenheim, Germany) was used to determine lymphocytes concentration **(Bleul *et al.*, 2002)**.

2.11. Statistical Analysis

Statistical analysis was performed using SPSS (Ver. 22.0 for Windows, IBM, Houston, TX, USA). Experimental results were expressed as mean ± SE. Statistical significance was tested with one-way ANOVA followed by a post hoc test, and p-values < 0.01 for IgG and IgM and <0.05 for the remaining variables were applied according to Steel and Torrie **(1980)**.

3. RESULTS AND DISCUSSIONS

3.1. chemical composition of Garden cress seeds (*Lepidium sativum*)

Garden cress (*Lepidium sativum*) seeds, which are edible whole, are known for their health-promoting properties. Therefore, it has been suggested that these seeds may be a functional food. Preliminary work was conducted on the chemical composition of the seeds and the possibility of using them as a nutraceutical food ingredient in dietary fibre formulations was explored. Whole seeds were analyzed for chemical composition (Table 1). The seeds were mainly composed of fibre; crude fibre reached 30.0 ± 1.60 %. Besides, the seeds contained considerable amounts of fat and protein, 27.6 ± 0.34% and 22.4 ± 0.23 %, respectively. The protein content of *Lepidium sativum* is equivalent to that of animal protein sources such as beef, poultry, and fish which contain 16-21, 17-23, and 16-24 %, respectively **(FNB, 2005)**. The carbohydrate content was 18.2 ± 1.11 %, and the ash content was 1.8 ± 0.14 %. The obtained results were comparable to those of Gokavi *et al.* **(2004)**.

The use of *Lepidium sativum* seeds as a dietary supplement in the human diet has already been recommended by other researchers because they contain a considerable amount of iron and calcium among other important elements. The presence of high macro and microelements and antioxidant properties would increase their use **(Gokavi *et al.*, 2004; Kasabe *et al.*, 2012; Sat *et al.*, 2013)**.

Table (1): Chemical composition of garden cress seeds (*Lepidium sativum*)

Component	
Protein %	22.40 ± 0.23
Fat %	27.60 ± 0.34
crude fibre %	30.00 ± 1.60
Carbohydrates %*	18.20 ± 1.11
Ash %	1.80 ± 0.14

Data are presented as mean ± SD

* carbohydrates calculated by the difference

3.2. Physicochemical analysis of milk

Analysis of full-fat buffalo milk used for fermented milk production was carried out by a milk analyzer. The percentages of fat, SNF, protein, lactose, and ash were 6.36%, 11.28%, 3.73%, 5.41%, and 1.70%, respectively (Table 2). Besides, the density of the milk was 1.0355 g/cm3 with a freezing point of - 0.530 °C; the milk had a pH of 6.7 at 4.5 °C.

Table (2): Chemical composition and physical properties of full-fat buffalo milk used in fermented dairy product making.

Component	Average value
Fat (%)	6.39 ± 0.01
SNF (%)	11.28 ± 0.01
Protein (%)	3.73 ± 0.02
Lactose (%)	5.41 ± 0.01
Density g/cm^3	1.0355 ± 0.00
Freezing point (°C)	-0.530 ± 0.00
Ash (%)	1.70 ± 0.00
pH	6.70 ± 0.00

Data are presented as mean ± SD

3.3. Fermentation time

The fermentation period for the control and fortified samples was observed (Table 3). It varied from 2 hours and 23 minutes to 3 hours and 21 minutes. The control had the lowest value, and samples with 3% *Lepidium Sativum* lasted the longest. The data showed that the incubation periods of fermented milk treatments changed gradually with the addition of *Lepidium Sativum*. Each 1% increase in *Lepidium Sativum* altered the fermentation time by 5-15 min (10 min on average). The incubation periods of *Lepidium Sativum* 1% were almost identical to those of the control.

3.4. Chemical composition and physical properties of the fermented dairy product

3.4.1. pH values

The pH of fermented dairy product samples containing 0 (control), 1, 2, and 3% ranged from 4.38 to 4.43. All samples had a similar pH to the control with a difference of ± 0.5 (Table 3). The most crucial process in making fermented milk is the acidification of milk, which largely regulates the chemical and rheological properties of the finished product. **(Dalgleish and Law, 1989; Lucey, 2004)**. When compared to the control, the administered treatments had no major impact on the final pH. Therefore, it can be stated that the fortification had a minimal effect on the pH of the finished product.

3.4.2. Viscosity

After production and overnight cooling, the viscosity of fermented milk treatments was tested. The range of values was between 1319 and 1999 mPa.S (Table 3). The viscosity of the control (1395 mPa.S) was the lowest to be recorded. The highest value (1999 mPa.S) was recorded in the presence of *Lepidium Sativum* 1%. Additionally, it was shown that supplemented samples had higher viscosity values than the control. Labropoulos *et al.* **(1984)** pointed out that the viscosity value of fermented milk is one of the most important characteristics, which is influenced by many factors, including the addition of fruits.

3.4.3. Diacetyl and acetaldehyde contents:

When compared to the control, the fortified treatments with *Lepidium Sativum* showed a progressive increase in acetaldehyde and diacetyl concentrations. (Table 6). In case the of acetaldehyde, the lowest concentration was found in the control (9.04 ppm). While the highest value was 10.16 ppm in the presence of 3% *Lepidium Sativum*. The same pattern was seen with diacetyl, where the control recorded 10.16 ppm and the samples containing 3% *Lepidium Sativum* achieved 11.49 ppm.

The data also indicated that when *Lepidium Sativum* was added in high concentrations, the levels of acetaldehyde and diacetyl in fermented milk samples slightly increased. This trend reflects a positive effect on the starter culture, indicating the probability of a prebiotic effect. This point needs further investigation to assess the prebiotic properties of *Lepidium Sativum*.

Table (3): Incubation periods, pH value, viscosity, acetaldehyde, and diacetyl concentration in control and supplemented fermented milk with *Lepidium sativum* at three concentrations (1, 2, and 3%).

Fermented milk treatment	Incubation period (h:min)	pH	Viscosity (mPa.S.)	Acetaldehyde (ppm)	Diacetyl (ppm)
Control	2:23	4.43 ±	1395 ±	9.04	10.16
***Lepidium Sativum* 1%**	2:35	4.41 ±	1998 ±	9.37	10.60
***Lepidium Sativum* 2%**	3:00	4.38 ±	1319 ±	9.71	10.71
***Lepidium Sativum* 3%**	3:21	4.38 ±	1827 ±	10.16	11.49

3.5. Sensory evaluation

The panellists evaluated every treatment according to four main criteria: Smell, taste, appearance, body and texture, and overall grade. Afterwards, scores for all treatments were recorded and the average scores were calculated. Table (4) demonstrates the sensory attribute scores of the control and the *Lepidium Sativum* fortified fermented (1, 2, and 3%). The evaluation of the smell of the samples showed that samples with *Lepidium Sativum* gained the highest scores, which was close to that of the control with an overall score of 4.5 points. The taste of the three treatments was described as creamy and acidic, in addition to the control. Control treatment received the highest score of 5.0 points, while the *Lepidium Sativum* treatments just scored 4.5 points.

All treatments produced well-formed like plain-yogurt fermented milk that was similar to the control in appearance (soft and thick), with scores of 4.0 and 3.5 points, respectively. Comparing the "body and texture" of samples revealed that samples containing *Lepidium Sativum* were thick, with a score of 3.5 for all treatments. The total acceptability overall grade for all samples ranged from 80 to 97%. The highest value was recorded for the control, while the samples containing *Lepidium Sativum* 1, 2, and 3% showed excellent overall scores of 90, 90, and 80%, respectively. No obvious differences in sensory attributes were found between the control and samples containing *Lepidium Sativum*.

Table (4): Sensory evaluation of plain and fortified fermented milk with *Lepidium Sativum* in three concentrations 1, 2, and 3%.

Fermented milk treatment	Smell		Taste		Appearance		Body and texture		Overall score 100%
	Score [a]	Description	Score [a]	Description	Score [a]	Description	Score [a]	Description	
Control	5.0	Yoghurt, milky	4.5	Creamy, Acidic	5.0	Soft, Thick	5.0	Homogenous	97
***Lepidium Sativum* 1%**	4.5	Yoghurt, milky	4.0	Creamy, Acidic	4.5	Soft, Thick	3.5	Light, Thick	90
***Lepidium Sativum* 2%**	4.5	Yoghurt, milky	4.0	Creamy, Acidic	4.5	Thick	3.5	Thick	90
***Lepidium Sativum* 3%**	4.5	Yoghurt	4.0	Creamy, Acidic	4.5	Thick	3.0	Thick	80

Score [a]: 1= bad, 2 = fair, 3= good, 4=very good, 5 = excellent

3.6. Parameters and Performance

Body Weight, final body weight gain (FWG g), daily weight gain (DWG g/D), feed conversion ratio (FCR), specific growth rate (SGR %/D), and relative growth rate (RGR %) in rats were monitored (Table 5 and Figure 1). The trials containing *Lepidium sativum* and *Bifidobacterium Longum* affected the rats' weight directly during the first week, and at the end of the experiment, a higher percentage of weight gain was observed in the treated groups. The most efficient treatment for restoring the weight of the rats was the use of *Lepidium sativum* at 3%. The low dose of *Lepidium sativum* (1%) improved the parameters the least compared to the groups fed with 2 and 3% *Lepidium sativum*. *Lepidium sativum* at concentrations of 1-3% had a dose-dependent relationship with weight gain.

Body weight records show a slight difference between control (–) and control (+) in favour of the first. However, all treatments containing *Lepidium sativum* and *Bifidobacterium longum* showed higher values than the controls. Final weight recorded 407, 396, 463, 452, and 456 g for control (–), control (+), 1%, 2%, and 3%, respectively. A similar trend was observed for FWG and DWG, where treatments fed with 1-3% had higher values ranged from 44 to 55g and 0.63 to 0.79 g/D, respectively. The obtained results reflect the higher impact of the treatments fed on the rats' weight gain.

FCR was found to decrease gradually throughout the experimental period (70 days) in all controls and treatments. However, a lower decrease in FCR was observed in all treatments with *Labidium Sativum* 1%, 2% and 3% and Bifidobacterium longum. In the case of control (–) and control (+), the decrease in FCR was 1.75 and 0.82, reaching 7.31 and 7.32, respectively. On the other hand, when *Labidium Sativum* 1%, 2% and 3% were added, the decrease in FCR was 0.86, 0.41, and 1.69 to reach 5.67 5.95, and 5.75, respectively. The obtained results reflect the

efficient enhancement in feed conversion. Comping the main findings from FWG, DWG, and FCR, it can be concluded that *Lepidium sativum* and *Bifidobacterium longum* enhanced feed utilization by 9.95 to 14.47%. In addition, the rats were able to prevent FCR from a rabid decrease, which was noticed in control (–). The trials containing *Labidium Sativum* 1%, and 2% were considered the best.

SGR (%/D) was used as a parameter to detect growth-limiting substances. The exponential growth phase is very short for a normal rat. SGR reaches its maximum early during growth, when cell density is low, and immediately decreases thereafter **(Ljunggren and Häggström, 1995)**. The values for SGR (%/D) and RGR (%) showed that *Labidium Sativum* 1%, 2% and 3% performed better than the controls. SGR and RGE were higher than control (–), 10.1 - 12.8 units and 19.85 - 23.85, respectively. Such data indicate that the treated groups were able to achieve better performance with the same amount of feed.

Table (5): Effect of *Labidium Sativum* 1%, 2% and 3% and *Bifidobacterium Longum* on final body Weight (FBW), Final Body Weight Gain (FWG), Daily Weight Gain (DWG), Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), and Relative Growth Rate (RGR) of Albino rats.

Treatment	FBW (g)	BWG (g)	DWG	FCR	SGR%/day	RGR%
control -	405.6±4.80^{b}	194.8±5.46^{b}	2.78±0.08^{b}	7.21±0.21^{a}	0.94±0.34^{b}	92.80±4.37^{b}
control +	396±9.82^{b}	192.6±7.81^{b}	2.75±0.11^{b}	7.32±0.30^{a}	0.95±0.02^{b}	94.58±2.95^{b}
Lepidium	463.4±12.52^{a}	247.8±7.76^{a}	3.54±0.11^{a}	5.67±0.17^{b}	1.09±0.02^{a}	115.05±2.91^{a}
Lepidium	452.2±15.97^{a}	237.8±11.27^{a}	3.41±0.16^{a}	5.95±0.31^{b}	1.05±0.03^{ab}	111.09±3.26^{a}
Lepidium	456.4±5.03^{a}	244.2±4.24^{a}	3.48±0.06^{a}	5.75±0.10^{b}	1.10±0.02^{a}	114.86±2.85^{a}

Data are presented as mean ± SD

Means with different superscripts (a-e) in the same column are statistically different ($p < 0.05$)

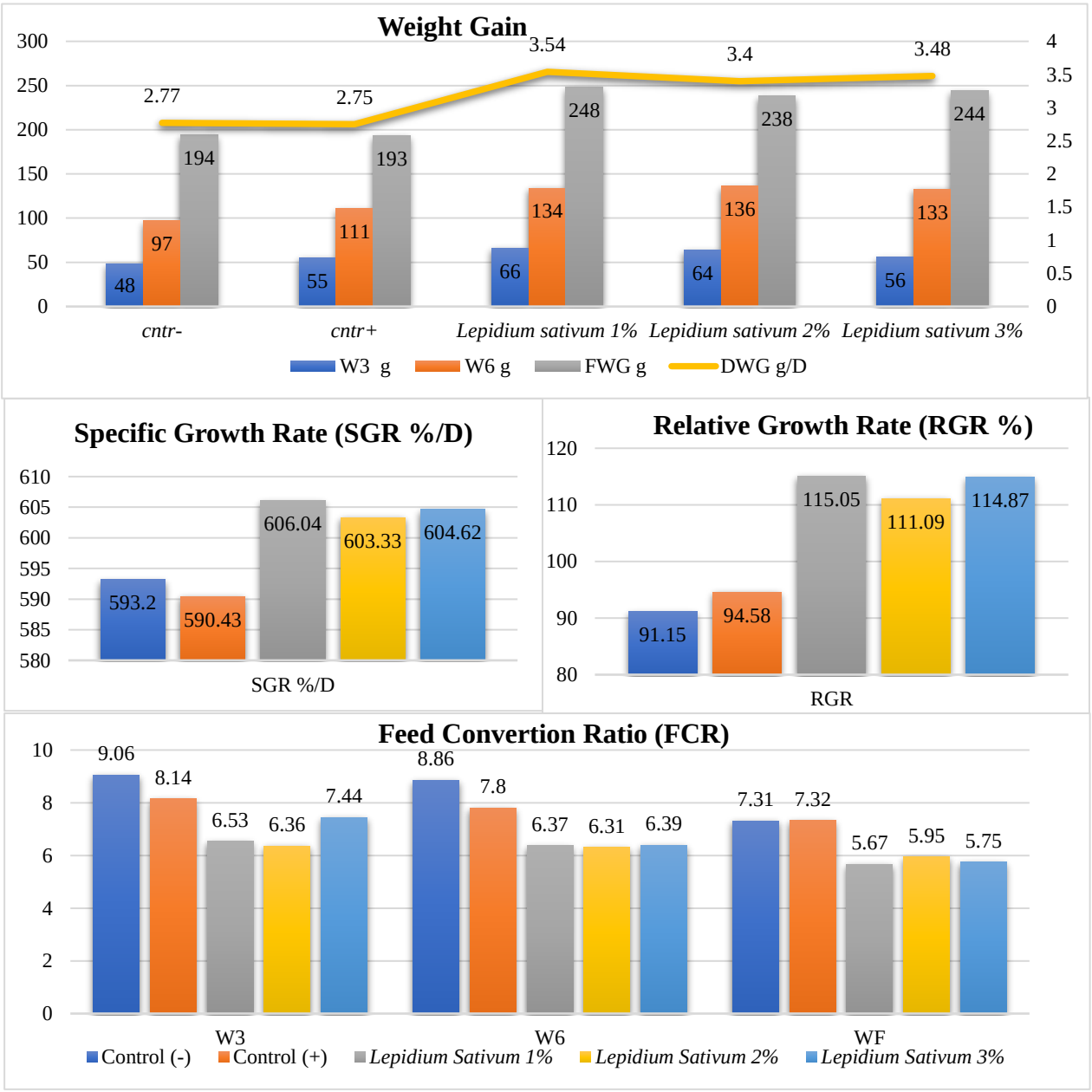

Figure (1): Final Body Weight Gain (FWG), Daily Weight Gain (DWG), Specific Growth Rate (SGR), Relative Growth Rate (RGR), and Feed Conversion Ratio (FCR) development of male albino rats through ten weeks (70 days). Wight (g) were measured four times: zero time (W0), after three weeks (W3), after six weeks (W6), and at the end of the experiment (WF). Rats are divided into 5 groups: control (-) basal feed, control (+); *Lepidium sativum* 1, 2 and 3% respectively.

3.7. Immunological measurements

Concerning IgG, no significant difference ($P \leq 0.01$) was observed among all groups. Normally, IgG needs more time to develop in comparison with other parameters, especially IgM **(Ishida *et al.*, 2006; Wang *et al.*, 2007)**. A significant improvement was found in IgM among all groups, except for control (+) and *Lepidium Sativum* 1%. Nevertheless, there was a significant difference between the control (-) and the control (+) trials in favour of the last. In the three groups treated with *Lepidium Sativum* 1, 2, and 3% and *Bifidobacterium Longum*, a gradual significant increase was recorded depending on the dose. The highest value was observed in the case of 3% *Lepidium Sativum* (61.33 ± 4.67) (Table 6). Results suggest that the addition of *Bifidobacterium Longum* has a positive effect on IgM in the case of control (+). Nevertheless, adding *Lepidium Sativum* 1, 2, and 3% triggered a stronger development of all IgM values in all treated groups. It can be concluded that the combination will enhance the host immune system. Further studies should be carried out to fully understand and determine whether higher doses would offer better performance.

The numbers of lymphocytes, monocytes, eosinophils, and neutrophils indicate that *Lepidium Sativum* in combination with *Bifidobacterium longum* alleviates immunity in a dose-dependent manner (Table 7).

Table (6): Effect of three different concentrations of *Lepidium sativum* (1, 2, and 3 %) on IgG and IgM of Albino rats after 70 days of growth.

Parameters Treatments	IgG (mg/dl)	IgM (mg/dl)
Control (-)	182.33 ± 3.84^{ab}	28.00 ± 2.52^{f}
Control (+)	171.33 ± 6.69^{ab}	31.33 ± 3.76^{ef}
***Lepidium Sativum* 1%**	179.67 ± 6.17^{ab}	31.67 ± 5.24^{ef}
***Lepidium Sativum* 2%**	189.33 ± 9.82^{ab}	39.67 ± 2.85^{bcdef}
***Lepidium Sativum* 3%**	189.00 ± 12.00^{ab}	61.33 ± 4.67^{b}

Data are presented as mean ± SD
Means with different superscripts (a-e) in the same column are significantly different ($P \leq 0.01$)
(IgG) Immunoglobulin G; IgM: Immunoglobulin M

Table (7): Effect of eighteen trials on some hematology parameters of treated male albino rats.

Treatment	Lymphocytes	Monocytes	Eosinophils	Basophils	Neutrophils
Control -	68.73	2.12	2.02	0	27.13
Control +	69.98	1.78	2.68	0	25.56
***Lepidium sativum* 1.0%**	75.99	2.89	1.76	0	19.36
***Lepidium sativum* 2.0%**	77.61	1.67	1.90	0	18.82
***Lepidium sativum* 3.0%**	79.33	1.55	2.24	0	16.88

CONCLUSION

Lepidium Sativum was used as a medicinal spice in the Middle East and was very popular there. A new functional dairy product containing *Lepidium Sativum* and *Bifidobacterium longum* was developed and tested for its nutritional and immunopotentiation properties in male albino rats. The product dose-dependently increased weight gain, SGR, RGR and FCR. IgM, lymphocyte, monocyte, eosinophil and neutrophil levels were improved. IgM was significantly increased in control (+), 1, 2, and 3%. The results of adding *Lepidium Sativum* and *Bifidobacterium longum* to fermented dairy products suggest that they may be useful and profitable for boosting immunity and defence against stress damage. Further studies should be conducted to fully understand the mechanism of action and to determine if higher doses would provide better performance.

REFERENCES

Aburjai T., Darwish R.M., Al-Khalil S., Mahafzah A. and Al-Abbadi A. (2001) Screening of antibiotic-resistant inhibitors from local plant materials against two different strains of Pseudomonas aeruginosa. Journal of Ethnopharmacology 76:39-44.

Asioli D., Aschemann-Witzel J., Caputo V., Vecchio R., Annunziata A., Næs T. and Varela P. (2017) Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. Food Research International 99:58-71.

Balthazar C.F., Guimarães J.F., Coutinho N.M., Pimentel T.C., Ranadheera C.S., Santillo A., Albenzio M., Cruz A.G. and Sant'Ana A.S. (2022) The future of functional food: Emerging technologies application on prebiotics, probiotics and postbiotics. Comprehensive Reviews in Food Science and Food Safety 21:2560-2586.

Bianchi M.E. (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. Journal of leukocyte biology 81:1-5.

Bishayee A. (2009) Cancer Prevention and Treatment with Resveratrol: From Rodent Studies to Clinical TrialsResveratrol and Cancer: In vivo and Clinical Studies. Cancer prevention research 2:409-418.

Bleul U., Bleul U., Sobiraj A. and Bostedt H. (2002) Evaluation of the Cell-Dyn 3500 Haematology Analyser for Bovine Blood. Comparative Clinical Pathology 11:201-210.

Bruce H.M. and Parkes A. (1949) Feeding and breeding of laboratory animals. IX. A complete cubed diet for mice and rats. Epidemiology & Infection 47:202-208.

Brunner G. (2005) Supercritical fluids: technology and application to food processing. Journal of Food Engineering 67:21-33.

Buckley R.H. (1986) Humoral immunodeficiency. Clinical Immunology and Immunopathology 40:13-24.

Cooper K.H. (2013) Aerobics program for total well-being: Exercise, diet, and emotional balance Bantam.

Costa M.R., Elias-Argote X.E., Jiménez-Flores R. and Gigante M.L. (2010) Use of ultrafiltration and supercritical fluid extraction to obtain a whey buttermilk powder enriched in milk fat globule membrane phospholipids. International Dairy Journal 20:598-602.

Dalgleish D.G. and Law A.J.R. (1989) pH-Induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. Journal of Dairy Research 56:727-735.

Dispenzieri A., Gertz M.A., Therneau T.M. and Kyle R.A. (2001) Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clinic Proceedings 76:476-487.

Eddouks M. and Maghrani M. (2008) Effect of Lepidium sativum L. on renal glucose reabsorption and urinary TGF-β1 levels in diabetic rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 22:1-5.

FNB. (2005) A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board.

Gaafar M., Marzouk S., Zayed H., Soliman L. and El-Deen A.S. (2013) Structural studies and mechanical properties of some borate glasses doped with different alkali and cobalt oxides. Current Applied Physics 13:152-158.

Gokavi S.S., Malleshi N.G. and Guo M. (2004) Chemical Composition of Garden Cress (Lepidium sativum) Seeds and Its Fractions and use of Bran as a Functional Ingredient. Plant Foods for Human Nutrition 59:105-111.

Hachimura S., Totsuka M. and Hosono A. (2018) Immunomodulation by food: Impact on gut immunity and immune cell function. Bioscience, Biotechnology, and Biochemistry 82:584-599.

Ishida T., Ichihara M., Wang X., Yamamoto K., Kimura J., Majima E. and Kiwada H. (2006) Injection of PEGylated liposomes in rats elicits PEG-specific IgM, which is responsible for rapid elimination of a second dose of PEGylated liposomes. Journal of Controlled Release 112:15-25.

Jain S., White M. and Radivojac P. (2016) Estimating the class prior and posterior from noisy positives and unlabeled data. Advances in neural information processing systems 29.

Kasabe P.J., Patil P.N., Kamble D. and Dandge P.B. (2012) Nutritional, elemental analysis and antioxidant activity of garden cress (Lepidium sativum L.) seeds. Int. J. Pharm. Pharm. Sci 4:392-395.

Labropoulos A.E., Collins W.F. and Stone W.K. (1984) Effects of Ultra-High Temperature and Vat Processes on Heat-Induced Rheological Properties of Yogurt. Journal of Dairy Science 67:405-409.

Lindsay R. and Day E. (1965) Rapid quantitative method for determination of acetaldehyde in lactic starter cultures. Journal of Dairy Science 48:665-669.

Ljunggren J. and Häggström L. (1995) Specific Growth Rate as a Parameter for Tracing Growth Limiting Substances, in: E. C. Beuvery, J. B. Griffiths and W. P. Zeijlemaker (Eds.), Animal Cell Technology: Developments Towards the 21st Century, Springer Netherlands, Dordrecht. pp. 229-233.

Lucey J.A. (2004) Cultured dairy products: an overview of their gelation and texture properties. International Journal of Dairy Technology 57:77-84.

Nagura T., Hachimura S., Hashiguchi M., Ueda Y., Kanno T., Kikuchi H., Sayama K. and Kaminogawa S. (2002) Suppressive effect of dietary raffinose on T-helper 2 cell-mediated immunity. British Journal of Nutrition 88:421-426.

Pack M.Y., Sandine W.E., Elliker P.R., Day E.A. and Lindsay R.C. (1964) Owades and Jakovac Method for Diacetyl Determination in Mixed-Strain Starters1. Journal of Dairy Science 47:981-986.

Sakai F., Hosoya T., Ono-Ohmachi A., Ukibe K., Ogawa A., Moriya T., Kadooka Y., Shiozaki T., Nakagawa H., Nakayama Y. and Miyazaki T. (2014) Lactobacillus gasseri SBT2055 induces TGF-beta expression in dendritic cells and activates TLR2 signal to produce IgA in the small intestine. PLoS One 9:e105370.

Sat I.G., Yildirim E., Turan M. and Demirbas M. (2013) ANTIOXIDANT AND NUTRITIONAL CHARACTERISTICS OF GARDEN CRESS (Lepidium sativum). Acta Scientiarum Polonorum Hortorum Cultus 12:173-179.

Soukoulis C., Panagiotidis P., Koureli R. and Tzia C. (2007) Industrial Yogurt Manufacture: Monitoring of Fermentation Process and Improvement of Final Product Quality. Journal of Dairy Science 90:2641-2654.

Steel R.G.D. and Torrie J.H. (1980) Principles and procedures of statistics, a biometrical approach McGraw-Hill Kogakusha, Ltd.

Taday B. and Eigenbrodt E. (1998) Entwicklung eines kompetitiven ELISA zur Immunglobulinbestimmung des Kalbes. Persönliche Mitteilung (aus Stengel KH, 1998).

Valero-Cases E., Cerdá-Bernad D., Pastor J.-J. and Frutos M.-J. (2020) Non-dairy fermented beverages as potential carriers to ensure probiotics, prebiotics, and bioactive compounds arrival to the gut and their health benefits. Nutrients 12:1666.

Wang X., Ishida T. and Kiwada H. (2007) Anti-PEG IgM elicited by injection of liposomes is involved in the enhanced blood clearance of a subsequent dose of PEGylated liposomes. Journal of Controlled Release 119:236-244.

William H. (2000) Official methods of analysis of AOAC international. AOAC official method 985.29.

الملخص العربي

تقييم الخصائص الغذائية والمعززة للمناعة للبن الوظيفي المخمر المحتوى علي بذور الشيا (*Lepidium sativum*) في ذكور الجرذان البيضاء

محمد أحمد السيد جمعة [1] ، أحمد ربيع عبده محمد عوض [2] ، خاطر عبدالفتاح أحمد خاطر [2] ، سمير عكاشة احمد شلبى [2]

1قسم علوم الغذاء ، كلية الزراعة سابا باشا ، جامعة الإسكندرية ، الإسكندرية ، مصر.

2قسم الألبان ، كلية الزراعة ، جامعة الأزهر ، القاهرة ، مصر.

تمت دراسة بعض التأثيرات التغذوية والمعززة للمناعة للبن وظيفي مخمر المحتوى على بذور الشيا في نموذج ذكور الجرذان البيضاء. تم قياس تأثير المنتج على زيادة الوزن اليومية DWG، معدل النمو النوعيSGR ٪ ، معدل النمو النسبي RGR ، نسبة تحويل العلف FCR ، الجلوبولين المناعي IgG و IgM ، lymphocytes, monocytes, eosinophils, basophils, neutrophils ، تم دراسة المتغيرات في خمس مجموعات تجريبية. بعد فترة تأقلم مدتها 21 يومًا ، أجريت التجربة لمدة 70 يومًا. أظهرت النتائج أن جميع الخصائص الحسية للمنتج كانت مقاربة للبن المتخمر القياسي وأظهرت قبولًا ممتازًا بنسبة 90٪. أثرت بذور الشيا *Lepidium sativum* بشكل كبير على وزن الفئران خلال الأسبوع الأول. وفي نهاية التجربة، لوحظ زيادة في الوزن في المجموعات المعالجة. كانت المعاملة المحتوية على 3% هي الأكثر فعالية. أظهرSGR ٪ و RGR و FCR نفس الاتجاه. تم تحسين IgM بشكل ملحوظ في جميع المجموعات باستثناء المجموعة الضابطة (+) والمجموعة التي عوملت بـ .1٪ *Lepidium sativum* في المجموعات الثلاث التي عملت بـ 1 و 2 و 3 ٪ و *Bifidobacterium longum* ، لوحظ زيادة معنوية تدريجية اعتمادًا على الجرعة. ولوحظت أعلى قيمة في حالة 3٪ بقيمة 61.33 ± 4.67. أشار عدد الخلايا lymphocytes, monocytes, eosinophils, basophils, neutrophils إلى أن *Lepidium sativum* مع *Bifidobacterium longum* يعزز المناعة وفقًا للجرعة. كانت 3٪ أكثر فعالية من 1 و 2٪ لمعظم المتغيرات، مما يشير إلى أن 3٪ *Lepidium sativum* مع التخمير بروبيوتيك عزز النشاط المناعي في ذكور الجرذان البيضاء.

الكلمات المفتاحية:

Lepidium Sativum؛ IgM. مفتش. الخلايا الليمفاوية؛ الحليب المخمرة؛ *Bifidobacterium longum* ؛ أداء النمو