إقـــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Effect of Kefir Intake on Growth Performance, and Some Biochemical Profiles Among Domestic Rabbits

تأثير تناول لبن الكفير على معدلات النمو، وبعض التحاليل البيوكيميائية لدى الأرانب المنزلية

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كلية العلوم
ماجستير العلوم الحياتية
علم الأحياء الدقيقة

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ **أسماء ابراهيم محمد القريناوي** لنيل درجة الماجستير في كلية *العلوم* قسم **العلوم الحياتية – أحياء دقيقة** وموضوعها:

تأثير تناول لبن الكفير على معدلات النمو، وبعض التحاليل البيوكيميائية بين الأرانب المنزلية

Effect of kefir intake on growth performance, and some biochemical profiles among domestic rabbits

وبعد المناقشة التي تمت اليوم الأحد 02 ربيع الأول 1437هـ، الموافق 2015/12/13م الساعة الحادية عشرة صباحاً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

أ.د. بكر محمود الزعبوط	مشرفاً و رئيساً	.....
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د. كمال العبد الكحلوت	مناقشاً داخلياً	.....
د. أيوب راغب الدلو	مناقشاً خارجياً	.....

وبعد المداولة أوصت اللجنة بمنح الباحث درجة الماجستير في كلية *العلوم*/ قسم **العلوم الحياتية – أحياء دقيقة**.

واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

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Dedication

IN MEMORY OF

MY FATHER

(1940-2006)

Acknowledgments

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Effect Of Kefir Intake On Growth Performance and Some Biochemical Profiles Among Domestic Rabbits

Abstract

Background: Kefir is a natural probiotic food. It contains a complex mixture of both bacteria, yeasts, many vitamins, minerals, amino acids, and enzymes. Also It contains numerous bioactive ingredients that give its unique health benefits, for instance, strengthening immune system**,** metabolism, improving anti-allergic resistance, antitumor activity, improving intestinal immunity, antimicrobial activity, regulation of cholesterol, improving sugars digestion and antioxidant activity.

Aims: The study aimed to investigate the effect of kefir intake on growth performance, and some biochemical profiles among domestic rabbits.

The study design: it was a case-control study.

Materials and method: Kefir starter was obtained from Mrs. Al Nagar who she is a Physician popular, Nusirat Camp, Slahdeen street. Experiment was carried out on the rabbits that lived in normal condition, they were divided into three groups (one control & two cases). All groups matched each other in age, initial body weight, and all other environmental conditions. The sample included 24 rabbits at of 35 - 40 days. Each group have 8 rabbits, first group is a control received normal drinking water. The case groups (T1 & T2) are the rabbits that were drunk water with 10% and 20% Kefir, respectively. All rabbits were individually weighed at the beginning of experiment then they were individually weighed weekly intervals until the end of the experiment. Feed consumption of each experimental unit was recorded weekly and feed conversion ratio was calculated. At the end of the study period, 2 rabbits were randomly selected from each group for slaughter and blood was collected for biochemical analysis. SPSS system (V20) was used to analyze the obtained data.

Results: The results of the study showed that total body weight gain were similar in control & T2 groups during whole the trial period ($p>0.05$). The

highest growth observed in rabbits that took 20% Kefir milk and The lowest growth observed in rabbits that took 10% Kefir milk at first 4 weeks of growth period. But when compared with control group, it was non-significant. The same results was clear after 6 weeks in growth of cases. Total average daily feed intake, feed conversion ratio were showed a significant decrease among cases compared to control group.

As Kefir concentration increased to 20% of water, there were significant decrease in skin weight, kidneys, spleen, lungs, internal body fats and liver. In contrast there were significant increase in Caracas, head and viscera weights. But when increased to 10% of water there were significant decrease in, internal body fats, viscera weights and liver. On the other hand there were significant decreases in fasting blood sugar, insulin growth factor1, low density lipoprotein, uric acid and free thyroxin as kefir percentage increased to 20%. In contrast these results, there were significant increased with total cholesterol, aspartate aminotransferase, alanine aminotransferase among groups.

Key words: Kefir, rabbits, Biochemical profiles, Growth.

Abstract in Arabic

تأثير تناول لبن الكفير

على معدلات النمو وبعض التحاليل البيوكيميائية في الأرانب المنزلية

الخلاصة

المعلومات الأساسية: الكفير هو غذاء بروبيوتيك طبيعي، يتكون من خليط معقد من البكتيريا والخمائر، و يحتوي على العديد من الفيتامينات والمعادن والأحماض الأمينية والإنزيمات .كما يحتوي على العديد من مواد بيولوجية نشطة تعطي فوائد صحية فريدة من نوعها، على سبيل المثال، تعزيز الجهاز المناعي و التمثيل الغذائي ويكسب الجسم مقاومة مضادة للحساسية و مضاد للأورام و تحسين مناعة الأمعاء و مضاد للميكروبات و تنظيم الكولسترول وتحسين هضم السكريات ومضاد للأكسدة.

الهدف: تهدف هذه الدراسة إلى التعرف على تأثير تناول لبن الكفير على نمو الأرانب المنزلية، وبعض التحاليل البيوكيميائية .

نوع الدراسة: دراسة تجريبية ضابطة .

الطريقة و الأدوات: تم الحصول على حبيبات الكفير من طبيبة شعبية من عائلة النجار تعيش في مخيم النصيرات، شارع صلاح الدين، أما الأرانب فمن السوق المحلية حيث اشتملت العينة على 24 أرنباً في عمر (35-40) يوما، قسمت إلى ثلاث مجموعات، ملائمة لبعضها البعض في العمر ووزن الجسم الأولي، وجميع الظروف البيئية الأخرى ، في كل مجموعة 8 أرانب. المجموعة الأولى هي المجموعة الضابطة والتي كانت تتناول ماء الشرب العادي وعلف العنبر ، أما المجموعات الأخرى فهي المجموعات التجريبية التي تم تغذيتها على نفس العلف، بالإضافة إلى 10٪ و20٪ لبن الكفير في مياه الشرب على التوالي .تم وزن جميع الأرانب بشكل فردي في بداية التجربة. وبعد ذلك تم وزنها بشكل فردي أيضا في نهاية كل أسبوع إلى انتهاء وقت التجربة، كذلك تم وزن كمية العلف المستهلكة لكل وحدة تجريبية أسبوعيا و حساب نسبة التحويل الغذائي . و في نهاية فترة الدراسة، تم اختيار 2 من الأرانب عشوائياً من كل مجموعة للذبح . حيث جمعت عينات الدم لإجراء التحاليل البيوكيميائية . ووزنت الأعضاء الداخلية للجسم. ولتحليل البيانات التي تم الحصول عليها تم استخدام نظام (V20, SPSS).

النتائج : أظهرت النتائج أنه لا توجد فروق ذات دلالة إحصائية (P> 0. 05)) في إجمالي الزيادة في وزن الجسم خلال الفترة التجريبية .ولكن من خلال الملاحظة، أفضل معدل وزن تم الحصول عليه كان بين الأرانب التي تم تغذيتها بلبن الكفير بنسبة 20% ,أما نسبة 10% فقد سجلت اقل المجموعات وزنا وذلك خلال الأسابيع الأربعة الأولى من فترة النمو، و بعد ستة أسابيع حصلنا على نفس النتائج في معدل الوزن بالمقارنة مع المجموعة الضابطة، مما يدل على عدم تأثر النمو بزيادة الفترة الزمنية .

وقد أظهر إجمالي متوسط استهلاك العلف اليومي و نسبة التحويل الغذائي انخفاضا كبيرا بين المجموعات التجريبية مقارنة بالمجموعة الضابطة . عند زيادة تركيز الكفير إلى 20 ٪ ، لوحظ انخفاض كبير في وزن الجلد والكلى والطحال والرئتين والكبد ودهون الجسم الداخلية .وفي المقابل لوحظ ارتفاع في معدل وزن اللحم والأحشاء. أما عند زيادة تركيز الكفير إلى 10% لوحظ انخفاض في وزن الأحشاء والكلى والطحال والرئتين والكبد واللحم و دهون الجسم الداخلية .وفي المقابل كان هناك ارتفاع في وزن الرأس. بينما لا توجد فروق ذات دلالة إحصائية في وزن الجلد والأرجل (P> 0. 05) بالمقارنة مع المجموعة الضابطة.

من ناحية أخرى أظهرت النتائج انخفاض كبير في نسبة السكر في الدم، هرمون النمو الشبيه بالأنسولين 1 البروتين الدهني منخفض الكثافة، حمض اليوريك وهرمون الثيروكسين الحر بارتفاع نسبة الكفير إلى 20% وعلى النقيض، كان هناك ارتفاع كبير في معدل الكولسترول الكلي و أنزيمات الكبد .(AST ,ALT) بينما لا توجد فروق ذات دلالة إحصائية (P> 0. 05)) في معدل البروتين الدهني عالي الكثافة، و اليوريا بين المجموعات الضابطة و التجريبية.

الكلمات المفتاحية: الكفير، الأرانب، التحاليل البيوكيميائية، النمو.

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Abbreviations

AST	**aspartate aminotransferase**
ALT	**alanine aminotransferase**
BUN	**Blood Urea Nitrogen**
FBS	**Fasting blood sugar**
FCR	**Feed conversion ratio**
FI	**Feed intake**
FRA	**Fodder Rabbits- Anbar**
FT4	**Free Thyroxin**
GH	**Growth hormone**
IGF-1	**Insulin growth factor hormone**
HDL - C	**high-density lipoprotein cholesterol**
LAB	**Lactic acid bacteria**
LDL - C	**low-density lipoprotein 'cholesterol**
VLDL	**Very -low density lipoprotein**
TSH	**Thyroid stimulating hormone**
TG	**Triglyceride**
TC	**Total cholesterol**

Chapter 1

Introduction

1.1 Overview

Kefir is a natural probiotic food. A probiotic is a live microbial food supplement, that beneficially affects the host animal, by improving the microbial balance and they are used in fermented dairy products (**Semih and Cagindi, 2003**).

It is a fermented milk, where it was discovered in the Caucasus regions. It is prepared by putting kefir grains in cow's milk, camel or goat in room temperature **(Pogacic et al., 2013).**

Kefir grains measure 1–3 cm in length, are lobed, irregularly shaped, they are white to yellow- white in color, look like small cauliflower florets and have a slimy but firm texture, figure1.1. Grains are kept viable by transferring them daily into fresh milk and allowing them to grow for approximately 20 hours **(Farnworth, 2005)**.

Figure 1.1: Kefir grains (Farnworth , 2005)

It is the product of milk fermentation with Kefir grains, which contain a complex mixture of both bacteria (including various species of lactobacilli, lactococci, leuconostocs and acetobacteria) and yeasts (both lactose-fermenting and non-lactose-fermenting) such that beneficial yeast as well as friendly probiotic bacteria found in yogurt. It is an old world food fermented

milk beverage that looks a little like yogurt. It can also be prepared from dairy alternatives such as coconut milk or soy milk **(Elinoar, 2008)**.

Kefir contains many vitamins, minerals, amino acids and enzymes. Particularly calcium, phosphorus, magnesium, B2 and B12, vitamin K, vitamin A, folic acid and vitamin D. Tryptophan, one of the essential amino acids abundant in kefir, is well known for its relaxing effect on the nervous system and others **(Gaware et al; 2011).** See Table1.1 that shows the Nutritional and Chemical composition of milk kefir.

Original Kefir contains numerous bioactive ingredients that give its unique health benefits, such as, for instance, strengthening immune system **(Celso et al., 2005),** metabolism, improving anti-allergic resistance **(Liu et al., 2006)**, antitumor activity, improving intestinal immunity, antimicrobial activity, regulation of cholesterol, improving sugars digestion and antioxidant activity **(Gorsek and Tramsek, 2011)**.

Bioactive ingredients in Kefir is exopolysaccharides that produced by a variety of lactic acid bacteria (LAB) including Lactobacillus, Streptococcus, Lactococcus and Leuconostoc. They have protective and adaptive properties on their bacterial producers **(Farnworth,. 2005).** Peptides formed during the fermentation process or during digestion such as Opioid peptides, Immunomodulatory peptides, Mineral binding peptides, Antithrombotic peptides, Antimicrobial peptides, Antihypertensive peptides and antioxidant peptides **(Shrikant et al., 2011)** have bioactive properties, and demonstrate a variety of physiological activities, including stimulation of the immune system in animal models.
(Thoreux and Schmucker, 2007).

Antibiotics treatment may kill the good bacteria in the large intestine. Kefir replenishes protective intestinal flora **(Thoreux and Schmucker**, **2007)**. It is known that it have antimicrobial agents properties.

Table1.1 Nutritional &Chemical composition of milk kefir during storage **(Dominic, 2015).**

Components	**Percent/100 gm**	**Minerals components**	**Milligram [mg] /100 gm**
Energy Fat Protein Lactose Water	61 K Cal 3.5 3.3 3.5 87.5	Calcium Phosphor Magnesium Potassium Sodium Chloride	120 100 12 150 50 100
Fatty-acid	**gram [gm]**	**Vitamins**	**milligram \ [mg]**
Milk acid	0.8	A	0.06
Pyruvic acid	a	Carotene	0.02
Hippuric acid	a	Thiamin	0.02
Orotic acid	b	B2	0.17
Citric acid	b	B6	0.05
Lactic acid	1.00	B12	0.005
Ethyl alcohol	0.9	Folic acid	0.0095
Butyric acid	c	Niacin	0.09
Palmiitic acid	c	C	1.00
Palmitoleic acid	c	D	0.08
Oleic acid	c	E	0.11
Cholesterol	0.005 - 0.1300		
Phosphates	0.04		
Essential Amino Acids	**gram [gm]**	**Trace Elements**	**milligram [mg]**
Tryptophan Phenylalanin+tyrosin Leucine Isoleusine Threonine Methionine + cystine Lycine Valine	0.05 0.35 0.34 0.21 0.17 0.12 0.27 0.22	Iron Copper Molybdenum Magnesium Zinc	0.05 0.012 0.0055 0.005 0.36
Aromatic Compounds			
Acetaldehyde	1.1g/100g		

a : non detected during storage. b: increase slightly during storage. c: higher concentrations in kefir, than that found in fresh milk and yogurt.

1.2 General objective

●The study aimed to evaluate the effect of Kefir intake on growth performance, lipid profile, functions of liver, kidney, thyroid gland, and glucose level among domesticated rabbits.

1.2 Specific objectives

1. To calculate growth rate and feed conversion ratio of the rabbits.
2. To determine Insulin growth factor hormone (IGF1) and glucose level in serum of the growing rabbits.
3. To determine total cholesterol, Triglyceride (TG), low density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) level in serum of rabbits.
4. To test effect of kefir intake on liver function and kidney function.
5. To examine effect of kefir intake on thyroid gland.

1.4 Significance of this study

● Kefir is considered as a probiotic that protect the body against many infectious diseases and strengthen the self-immunity.

● According to my knowledge, The usage of kefir in Gaza strip in making yogurt have not been carried out before. So, I would like to shed light on such materials and make sure their impact beneficial to public health.

● I would like the use of Kefir become common in Gaza Strip because of its possibility of its benefit in the treatment of some diseases and prevention.

Chapter 2

Literature review

2.1 Kefir

2.1.1 History of Kefir

Kefir is a word with a Turkish origin “keyif” means pleasure and “kopur” means milk or froth. Kefir is originated in the Caucasus Mountains more than 2000 years ago and is the oldest known fermented milk yoghurt. The secret of the Kefir grains, passed down from generation to generation, was considered a source of family and tribal wealth. For the ancient shepherds of the Caucasus who originally discovered Kefir, then, “Kefir” was a pleasurable, frothy milk drink. The shepherds stumbled upon kefir because they carried milk with them in leather pouches. When the milk would ferment, it would become an effervescent and tasty drink. In the early twentieth century, The Russian Physicians' Society contracted two cheese makers, the Blandov's brothers, to go out and search for the secret of Kefir, With help from the Blandov's brothers and The Russian Physicians' Society, In 1908 Irina Sakharova was famed with bringing the first batch of Kefir grains into Moscow where it was used, medicinally at first, in health sanatoriums as part-treatment for tuberculosis and other ailments with great success. In the last few decades, Kefir has made its way west **(Gaware *et al.*, 2011).** Kefir was used in former Soviet Union hospitals to treat conditions such as digestive disorders, cancer, tuberculosis, and even atherosclerosis **(Elinoar, 2008).**

2.1.2 The microbial population of Kefir grains.

Three main genera make up the bacterial population of Kefir: lactobacilli, lactococci, and leuconostoc. A fourth genus, acetobacter, is also often mentioned, but its presence is not reported by all research teams studying Kefir- could be a contaminant **(Farnworth, 2008).**

The microbial population of Kefir grains consist of lactic acid bacteria, acetic acid bacteria, yeasts, filamentous moulds and possibly other microorganisms which develop a complex symbiotic community. These microorganisms are agglutinated with a water-soluble polysaccharide (Kefiran) **(Santos et al., 2003; pogacic et al., 2012). figure 2.1** Kefiran has been studied extensively and has demonstrated anti-inflammatory and immunomodulating properties in animal and human trials. It has also demonstrated antibacterial and anti-mycotic properties **(Elinoar, 2008).**

A crude analysis of the grains shows that they are a mass of bacteria, yeasts, polysaccharides, and proteins with a chemical composition of 890 to 900 g/kg water, 2 g/kg lipid, 30 g/kg protein, 60 g/kg sugars, and 7 g/kg ashes . A study of the proteins in kefir grains using SDS-PAGE on acrylamide gels indicated that the major grain proteins had a higher molecular weight than milk proteins, indicating that they were not proteolysis products **(Farnworth, 2008).**

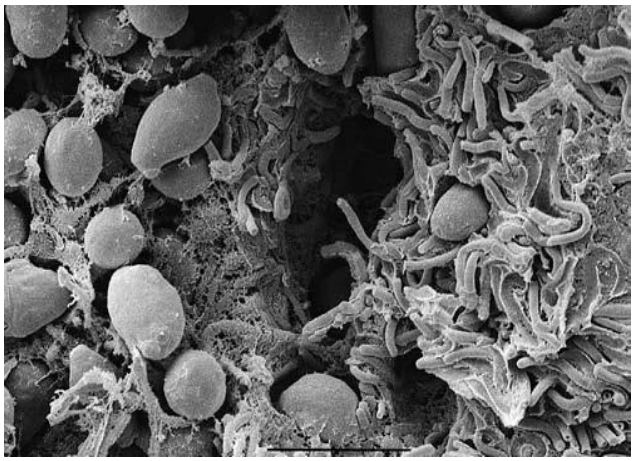

Figure 2.1 Electron micrograph of kefir grains showing bacteria and yeasts in carbohydrate or protein matrix. Magnification X 2555, bar indicates 10 µm (Farnworth and Mainville, 2003).

The microorganism profile of the final product does not necessarily parallel that of the grains because of conditions (pH and other) during the fermentation process. Also, the location of the microorganisms in the grains may be a factor. Yeasts are generally found in the interior of the grains, whereas the lactococci are found on the exterior **(Figure 2.2).** Therefore, the

number of yeasts found in the final product is lower than those counted in the grains themselves, whereas lactococci are numerous in the final drink. In many fermented milk products, the growth of several bacteria isolated from kefir grains is improved when yeast extract is added to the growth medium, indicating that the yeasts found in kefir grains are essential to maintain the integrity and viability of the micro flora population. Vitamins, amino acids, and other essential growth factors for bacteria are produced by yeasts, whereas bacterial metabolic end products are used as energy sources by yeasts **(Farnworth, 2008).**

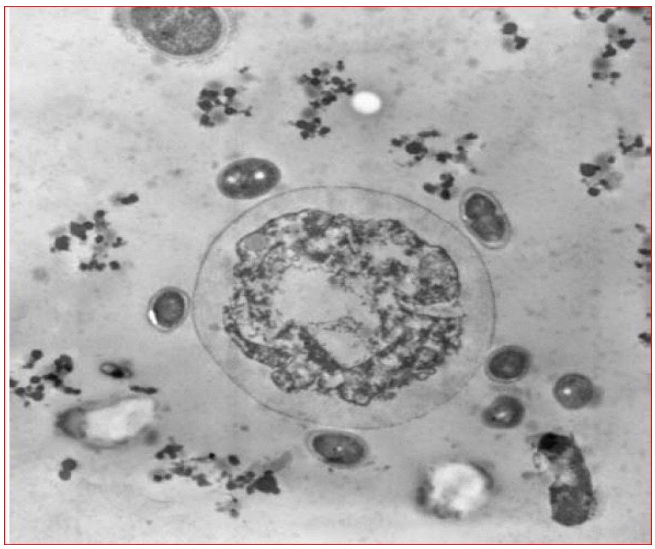

Figure.2.2 Electron micrograph of kefir showing symbiosis between bacteria and yeast. (Farnworth, 2008).

Interactions of yeasts with Lactic acid bacteria in Kefir milk fermentations may result in inhibition or elimination of undesirable microorganisms. It is believed that a symbiotic relationship may occur when LAB produce organic acids such as lactic acid which lower the pH. The lower pH, (~4.2- 4.6) being favorable for growth of many yeast species, causes the yeasts to become competitive in the immediate medium. Due to the low pH, the inhibitory metabolites produced, and the strong competitive effects of yeast and LAB populations. many spoilage and pathogenic microorganisms are inhibited, As a result. the shelf life of the fermented milks is extended **(Lefoka, 2009).**

The symbiosis found in the Kefir grain microorganism population allows the Grains to maintain uniformity so that throughout the year the microboilogI-

cal profile of Kefir grains and the Kefir drink remain stable in spite of variations in milk quality and the potential presence of antibiotics and other inhibiting substances **(Farnworth, 2008).**

Lactic acid bacteria organisms are ferment carbohydrates to form chiefly lactic acid. They have gras status and play an essential role in food fermentation given that a wide variety of strains are employed as starter cultures (or protective cultures) in the manufacture of dairy, meat, and vegetable products. The most important contribution of these microorganisms is the preservation of the nutritional qualities of the raw material through extended shelf life, the inhibition of spoilage and pathogenic bacteria. This contribution is due to competition for nutrients and the presence of inhibitor agents produced, including organic acids, hydrogen peroxide ,and bacteriocins (Bio preservation), an ecological approach to improve the safety and shelf-life of foods **(Ananou et al., 2007).**

In contrast, raw milk pH value is approximately 6.5 - 7.2 that a suitable environments for the majority of pathogenic microorganism initially dominant Gram-positive mesophilic aerobic bacteria then are replaced by Gram-negative and Gram-positive psychrotrophic bacteria when milk is cooled. They have the ability to produce heat stable extracellular and / or intracellular hydrolytic enzymes. Many of these enzymes retain their activity even after the conventional heat treatment of milk **(Samarzija et al,. 2012).**

2.1.3 Health Benefits of Kefir.

Many of references were written about Health Benefits of Kefir **(Semih and Cagindi, 2003; Gaware et al., 2011; Baltuska, 2013; Porteus, 2014; Moses and Deeseenthum, 2015). such as:**

• Skin care: Kefir is a natural anti-oxidant. Therefore, it keeps the skin youthful and glowing. It prevents acne, psoriasis and wrinkles.

• Brain-enhancement: One of the important kefir health benefits is that it can enhance the functioning of the brain. It is considered as a brain-food and

helps fighting the stress. It also improves the focus, reflexes and memory-retention power of the brain.

• Digestion: it improves the digestion, preventing constipation and helps in cleansing the intestines and regularizing the bowel movements.

• Heart health: Kefir also helps in maintaining the health of the heart, by clearing the vessels of the body and also regulate the blood pressure.

• Respiratory system Lungs: It cures the respiratory problems like tuberculosis. It plays a vital role in the treatment of bronchitis and asthma.

• Weight loss People: It has probiotics which speeds up the body's metabolism. This, in turn, burns the fat quickly, leading to weight loss.

• Stress-buster: It is said to be an excellent stress-buster. It is detoxify body and relax it.

• The Lactose Intolerant: Regular consumption of kefir helps people who lack the lactase enzyme to digest dairy products again. Some of the bacteria contained by Kefir helps to break lactose down.

• Heal Immune System: Kefir makes the body more efficient at destroying harmful pathogens, including harmful bacteria and viruses. In addition, the friendly bacteria in kefir can help destroy tumor cells.

• Prevents against ageing: Kefir is rich in antioxidants which help the aging process to slow down by neutralizing the free radicals by oxidizing them and reducing the impact of the damage caused to the body cells and tissues due to them.

• Antibiotic and antifungal: Kefir has certain anti-fungal properties; it proves helpful in treating conditions like psoriasis, candidiasis (yeast infection) and eczema. It may be useful in similar conditions, candidiasis (yeast infection), heart disease and HIV / AIDS.

• Anticancer agent: Kefir can inhibit the growth of cancerous cells and can prevent certain type of cancers like colon cancer, breast cancer. and reduce the size of tumors.

• Anti-inflammatory Agent: Kefir is also beneficial in treating a number of disorders like pancreatitis, gastritis, irritable bowel syndrome (IBS) and ulcers. Treats gum related diseases like periodontitis and cures bad breath. Beneficial in treating bone related disorders like arthritis, gout, rheumatism and other inflammatory diseases.

• Anti-Diabetic Agent: Kefir is beneficial for diabetics as it reduces the level of glucose in the blood and maintains the normal blood sugar level.

• Provides vitamins to the body Kefir contains rich amounts of vitamins like vitamin K, the B vitamins and important minerals like magnesium and calcium. These minerals and vitamins are important nutrients required by the body and regulate every internal organ in a proper manner.

• Reduces the levels of cholesterol: kefir helps to reduce high cholesterol levels. It is thus beneficial for preventing the occurrence of many cardiovascular diseases like heart attack and stroke.

• Prevention against toxins: Kefir plays an important role in protecting the body against the harmful effects of radiation and other toxic pollutants. It thus helps to enhance the immune function. Regular intake of kefir protects against the ill effects of ageing and helps to look younger **.**

2.2 Probiotics Food

Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host **(UNFAO/WHO, 2002).** The concept of probiotics evolved from a hypothesis first proposed by Metchnikoff, who believed that when consumed, the fermenting bacillus (*Lactobacillus*) positively influenced the micro flora of the colon, decreasing toxic microbial activities. He also concluded that the general human being's health is function of the balance between beneficial "good" probiotic bacteria and disease-causing "bad" bacteria in human gut **(Sanders, 1999; Awaisheh, 2012).**

The bacteria in the gut have several beneficial functions such as inhibiting the growth of pathogenic bacteria, aiding in digestion, and B vitamin

synthesis. There are over 500 different types of beneficial bacteria. Most bacteria, including LAB and probiotic bacteria are resistant to some antibiotics. Good bacteria can be wiped out by the use of antibiotics, stress, poor diet, or by the ingestion of pathogens **(Amor, 2013).** Table 2.1 showed key genera and species of microbes studied and used as probiotics.

Probiotics commonly are isolated from human and animal intestinal tracts. Probiotic products is Yogurt that perhaps the most common probiotic-carrying food. Earliest types of probiotic food were Cheese, milks made by LAB and fungal fermentation, fermented and unfermented milks Kefir, juices, smoothies, cereal, nutrition, and infant/toddler. In addition to being sold as foods, probiotics are sold as dietary supplements, medical foods, and drugs. Often these products are composed of concentrated, dried microbes packaged into capsules, tablets, or sachets. This format is convenient for the delivery of large numbers of microbes **(Awaisheh, 2012).**

Probiotics are resistance to stomach acid and pancreatic secretions such as bile and digestive enzymes would be important for probiotics needing to survive in high numbers through the small intestine. Beneficial effect, nonpathogenic, nontoxic, and free of significant adverse side effects retain stability during the intended shelf life of the product, contain an adequate number of viable cells to confer the health benefit **(Cast, 2007).**

Probiotics might provide several benefits, according to the National Institute of Health. However more research is needed to confirm their effectiveness and safety. Probiotics may treat diarrhea, especially following the use of antibiotics, reduce Symptoms of irritable bowel syndrome and inflammatory bowel syndrome such as Cohn's disease, Promote regularity, decrease lactose intolerance, improve serum cholesterol levels, decrease the risk of certain cancers, modify gut immune response and improve its barrier functions, control or reduce the development of certain allergies. reduce or shorten the risk of certain intestinal infections **(Adams, 2011).**

Table 2.1 Key genera and species of microbes studied and used as probiotics (Cast, 2007).

Genus	Species
Lactobacillus	***acidophilus*** ***brevis*** ***delbrueckiia*** ***fermentum*** ***gasseri*** ***johnsonii*** ***paracasei*** ***plantarum*** ***reuteri*** ***rhamnosus*** ***salivarius***
Bifidobacterium	***adolescentis*** ***animalisb*** ***bifidum*** ***breve*** ***infantis*** ***longum***
Streptococcus	***Thermophilus salivarius***
Enterococcus	***Faecium***
Escherichia coli	
Bacillus	***coagulansc clausii***
Bifidobacterium	***adolescentis***

The compliment to a probiotic is a prebiotic. The Prebiotics are non-digestible foods that make their way through our digestive system and help good bacteria grow and flourish. Prebiotics provide the non-digestible carbohydrates for probiotics. Prebiotics help feed and keep beneficial bacteria healthy **(Amor, 2013).**

2.3 Growth in animals

Growth in animals is defined as accretion of protein, fat, and bone. Growth is measured as the change in live weight or mass. Growth in animals affects by some factors such as; plane of nutrition, hormonal status, and

environmental conditions. Full live weight is measured without / withholding feed or water thereby, it varies during a day due to patterns of feed and water intake **(Owens et al., 1995).**

2.4 Biochemical parameter

During growth some metabolite compounds are synthesized and other break down. The liver plays a major role in break down and synthesis of cholesterol, it synthesis most of proteins found in blood including albumin, coagulation proteins and bile, uses enzymes and proteins. Synthesizes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). They are the most frequently utilized and specific indicators of hepatocellular necrosis. These levels increase in the serum with the death of hepatocytes either by necrosis or apoptosis **(Ahn, 2011).**They catalyze the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid respectively in gluconeogenesis. ALT is primarily localized to the liver but the AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver, pancreas, lungs, leucocytes, and red cells. Whereas the AST is present in both the mitochondria and cytosol of hepatocytes, ALT is localized to the cytosol, that is found in its highest concentrations in the liver and is more specific to the liver **(Limdi and Hyde, 2003; Thapa and Anuj, 2007)**.

Also some proteins are degraded. Creatinine is a waste product due to the normal breakdown of muscle tissue. It's filtered through the kidneys and excreted in urine. Creatinine level in the blood reflex the kidney function. The kidneys' ability to handle its called the Creatinine clearance rate, which helps to estimate the glomerular filtration rate (GFR) - the rate of blood flow through the kidneys.

Urea is a small molecule that is produced in the liver from protein that eaten. It is normally put out by the kidneys, so blood levels rise as kidneys fail. However other things change the level of urea in your blood too, so that it is not a simple guide to kidney function. But it is still a very useful test when used together with Creatinine **(Turner et al., 2004).**

Uric acid is produced from the natural breakdown of body's cells and from the foods. Most of the uric acid is filtered out by the kidneys and passes out of the body in urine. A small amount passes out of the body in stool. But if too much uric acid is being produced or if the kidneys are not able to remove it from the blood normally, the level of uric acid in the blood increases. High levels of uric acid in the blood can cause solid crystals to form within joints. This causes a painful condition called gout **(Poinier and Shadick, 2014).**

Thyroid hormones are secreted into the blood and then carried to every tissue in the body. They have an effect on growth and development of animal. These hormones are in correlation with metabolism of protein, carbohydrate and fat. Therefore, reduced thyroid secretion. will ultimately results in reduced metabolism of such nutrients **(Shaker, 2014).**

Thyroid hormone helps the body to use energy, stay warm and keep the brain, heart, muscles, and other organs working as they should. The measuring TSH level in a blood is the best way to initially test thyroid function, high TSH level indicates that the thyroid gland is failing. In most healthy individuals, a normal TSH value means that the thyroid is functioning normally **(American Thyroid Association, 2012).**

Lipid profile is A pattern of lipids in the blood. A lipid profile usually includes the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and the low-density lipoprotein (LDL) cholesterol.

Triglycerides are a type of fat the body uses to store energy and give energy to muscles. It is the most common type of lipid formed in animals. Triglyceride levels vary quite a bit over short time periods. A meal high in sugar, fat, or alcohol can raise the triglyceride level drastically, so the most repeatable measures of this lipid are taken after 12 hours of fasting. Even though sugar and alcohol are not lipids, The body will convert any form of excess calories into triglycerides for long-term storage. Only small amounts are found in the blood **(Rakesh, 2012).**

Cholesterol is a necessary molecule in human metabolism. It is a component of cell membranes, and is a building block of bile, estrogen and testosterone. The cholesterol necessary for normal metabolism is manufactured by the liver. It is present in the blood in three forms. It is a steroid lipid and insoluble in water. It is transported through the blood attached to a soluble protein, called a lipoprotein, present in the blood in three forms: Low density lipoprotein (LDL). This form contains the highest amount of cholesterol, so called “bad cholesterol”. High density lipoprotein (HDL) is called “good cholesterol”, that is packaged for delivery to the liver, where the cholesterol is removed from the body. Very low density lipoprotein (VLDL) that contains the highest amount of triglyceride. High VLDL cholesterol level lead to the buildup of cholesterol in arteries and increases the risk of heart disease and stroke **(Rakesh, 2012).**

Insulin growth factor hormone (IGF-1) is an important growth hormone, mediating the anabolic and linear growth promoting effect of pituitary GH protein. It has a GH independent growth stimulating effect, which with respect to cartilage cells is possibly optimized by the synergistic action with GH. It is secreted by many tissues and the secretary site seems to determine its actions. Most of it is secreted by the liver and is transported to other tissues, acting as an endocrine hormone. Also secreted by other tissues, including cartilaginous cells, and acts locally as a paracrine hormone **(Laron, 2001).**

Table 2.2 summarizes blood chemistry values for rabbits. Much of the obtained data comes from laboratory rabbits, kept in conditions that differ from those of house rabbits. Further parameters that influence blood chemistry are diet, husbandry, breed, age, sex, health condition and metabolic activity, indoor or garden rabbit. These values stated in **table 2.2** represent a reference range and should never be interpreted rigidly **(medirabbit.com, 2015).**

Table2.2 Blood chemistry values for rabbits.
http://www. /EN/Hematology/blood_chemistry.htm

Analyzed parameter	***Abbreviation***	***Value***	Units
Urea		***9.1 – 25.5***	mmol/l
Uric acid		***1 – 4.3***	mg/dl
Cholesterol		***0.1 – 2.00*** ***10 – 80***	mmol/l mg/dl
Creatinine		***53 – 124*** ***0.5 – 2.6***	mmol/l mg/dl
Glucose	**GLU**	***4.2 – 8.9*** ***75 – 140***	mmol/l mg/dl
Serum lipids		***150 – 400***	mg/dl
Thyroxin	**T4**	**82.37-106.82** **6.4 – 8.3**	nmol/l mg/dl
Triglycerides	**TG**	***1.4 – 1.76***	mmol/l
Alanine aminotransferase	**ALT**	***55 – 260***	IU/l
Aspartate aminotransferase	**AST**	***10 – 98***	IU/l

2.4 Previous studies

Marie-Pierre et al., (2002) reported that Kefir had no effect on total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglyceride concentrations nor on cholesterol fractional synthesis rates. No significant change on plasma fatty acid levels was observed with diet. However, both kefir and milk increased ($p < 0.05$) fecal isobutyric, isovaleric and propionic acids as well as the total amount of fecal short chain fatty acids. Kefir supplementation resulted in increased fecal bacterial content in the majority of the subjects.

Denli et al., (2003) determined the effects of the supplementation of separate probiotic (protexin), including organic acid combination, plant extracts, mineral salts (genex) and antibiotic (flavomycin) to broiler diets on performance, abdominal fat weight, abdominal fat percentage, liver weight, intestinal weight, intestinal length, intestinal pH, carcass weight, carcass yield of broiler chicks. The results obtained in the experiment showed that the group receiving 0.15% flavomycin + 0.2% genex supplemented in the basal diet was exhibited higher body weight gain, feed intake and carcass weight and better feed efficiency respectively than the control and other groups.

Je-Ruei Liu et al., (2006) study aimed to evaluate the hypocholesterol-aeic property of milk-kefir and soya milk-kefir. Male hamsters were fed on a cholesterol-free or cholesterol-enriched diet. The soya milk, milk-kefir and soya milk-kefir diets all tended towards a lowering of serum triacylglycerol and total cholesterol concentrations, and a reduction of cholesterol accumulation in the liver.

Moreover **Urdaneta et al., (2007)** reported data on the effects of kefir on enzymes and proteins present in the intestine. Food intake and body weight were recorded daily. The glucose, uric acid, cholesterol, triacylglycerols, and alkaline phosphatase activity were measured in the serum. No significant differences were found in the weight of the organs examined. An intestinal enzymatic analysis was carried out, and the results showed an increase of this activity in addition to the uptake of D- galactose by brush border membrane vesicles. This findings indicated that Kefir, in the conditions studied, could benefit protein digestion and reduce glycemic index.

Cenesiz et al., (2008) investigated the effect of varied amounts of Kefir applied in drinking water in relation to changes in total cholesterol serum, total lipid, (AST) and (ALT) activities in broiler chicks. At the end of experiment, live weights of the groups were significantly increased compared to that of the control group. Total cholesterol serum and total lipid levels were significantly reduced compared to that of in control group in response to kefir treatment. Moreover, kefir treatment in the groups did not result in any changes in serum AST and ALT activity. The obtained results demonstrated that use of kefir as

a probiotic in drinking water increases live weight, lowers total cholesterol and total lipid thus suggesting that its use in human diets may have beneficial effects.

Sahin and Yardimci (2009) studied the effects of Kefir as a probiotic on growth performance and carcass characteristics in Geese. The results showed that total body weight gain, total feed intake, feed conversion ratio values were similar in all experimental groups during whole the trial period. Despite the numerical variations, no statistical difference was seen among the groups in terms of slaughter traits, organ weights, carcass characteristics and meat composition values. On the other hand, a gradual increase was seen in abdominal fat amount contrary to the decrease in total skin amount based on the increased Kefir rates. Similarly, the numerical increase in meat weight opposite to the decrease in fat weight attracted attention.

Atasoglu et al., (2010) investigated the effect of kefir as a probiotic on the performance of goat kids during the pre- (45 days) and post-weaning (45 days) periods. The supplementation of different probiotics did not have any significant effect throughout the study on live weight and weight gain of the kids as compared to the Control group. The results of the study indicated that supplementation of kefir as a natural probiotic or a commercial probiotic source does not improve performance of goat kids under the conditions of that study and suggested that new approaches are required for studying the efficacy of this probiotic.

Aller et al., (2011) evaluated the effects of an acute treatment with a mixture containing 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* per day in patients with nonalcoholic fatty liver disease (NAFLD) improved liver aminotransferase levels in patients with NAFLD.

Dale, (2012) reported that drinking kefir daily will give hypothyroidism patient's system the re-balancing it needs to absorb Synthroid correctly. Synthroid is a hormone replacement therapy that attempts to make up for under-active thyroid. In addition to that benefit kefir can also help remedy some of the symptoms of hypothyroidism. These include acid reflux,

constipation, and gas. When they have a healthy and properly functioning digestive system these symptoms are no longer a problem.

Erkan et al., (2012) examined the effects of different doses of kefir on growth performance and oxidant-antioxidant status in the blood and liver tissues of Coruh trout, *Salmo coruhensis*, in different periods. There were no significant differences in specific growth rate, feed conservation rate, condition factor among fish. The data obtained from this experiment indicated that the same dose of kefir was more effective at the end of 3-month treatment than 2-month treatment. Although there was no statistical difference among groups, an increase in the glutathione peroxidase enzyme activity was observed in all groups compared to control groups. While catalase activity decreased in all groups compared to control group. It was concluded that kefir could play an antioxidant role and its effectiveness depended on dosage and time of application in *Coruh trout, S. coruhensis.*

Jascolka et al., (2013) investigated the effects of brown sugar-fermented kefir solution on the associate risk factor and development of atherosclerosis on mice. The results showed that in Kefir group, HDL increased and triacylglycerols decreased significantly, as compared to the control group. Lipid peroxidation and catalase activity were also reduced in liver of Kefir supplemented mice. Kefir supplementation, despite increasing HDL-c, was not associated to reductions of oxidized lipoproteins or atherosclerosis development. Kefir Supplementation improved lipid profile and oxidative stress but did not reduce atherosclerotic lesion.

Michel et al., (2013) investigated the effect of kefir and banana pulp and skin flours on the serum levels of total cholesterol, HDL-c, LDL-c and triacylglycerols in rats fed cholesterol-rich diet. They found that the fermented kefir reduced significantly the levels of VLDL, LDL-c and triacylglycerols, in addition to having increased HDL-c.

Ranganathan et al., (2013) studied the effect of Probiotic (Renadyl™) in stage 3 and 4 chronic kidney disease patients to confirm the safety and tolerability of several doses of Renadyl™. and were to quantify quality of life improvement, to confirm efficacy in reducing commonly known uremic toxins,

and to investigate the effects on several biomarkers of inflammation and oxidative stress. Statistically significant improvements were noted in Creatinine, C-reactive protein, hemoglobin, and physical functioning. Trends toward reduction were noted in blood Urea Nitrogen and pain. Other markers of inflammation and oxidative stress exhibited a lot of variation. The study did not have sufficient statistical power to ascertain changes in other molecular toxins

Reza et al., (2013) were conducted to examine the effects of Intermittent feeding programme and *Bacillus subtilis* based probiotic addition in diet on liver malic enzyme and isocitrate dehydrogenase activity, lipid metabolism and performance in broiler chickens**.** Body weight gain was not significantly different among any of the treatments, the birds raised under Intermittent feeding programme consumed significantly lower feed. Carcass weight as a percentage of live weight was not affected by probiotic Supplementation on the diet, All serum lipid metabolites concentration decreased with probiotic treatment.

Salaj et al., (2013) evaluated the effects of the different probiotic strains, *Lactobacillus plantarum* LS/07 and *Lactobacillus plantarum* Biocenol LP96, on lipid metabolism and body weight in rats fed a high fat diet. The results showed that *Lactobacillus plantarum* LS/07 reduced serum cholesterol and LDL cholesterol, but *Lactobacillus plantarum* Biocenol LP96 decreased triglycerides and VLDL, while there was no change in the serum HDL level and liver lipids. They have no significant change in body weight, gain weight, and body fat.

Judiono et al (2014) studied the effects of clear Kefir on bio molecular aspects of glycemic status of type 2 diabetes mellitus patients in Bandung, West Java [Study on Human Blood Glucose, C Peptide and Insulin]. The results showed that Supplementation of clear Kefir reduced blood glucose levels and Insulin and increase C Peptide.

In this study **Piccolo et al., (2015)** investigated the effects of dietary probiotic *Lactobacillus plantarum* on the growth performance, gut colonization and blood biochemical parameters of European sea bass (*Dicentrarchu-slabrax*). Dietary probiotic did not affect the growth performance of sea bass, but reduced mortality. Lactobacilli population in intestinal content of European sea bass was not statistically modified by the treatment. Fish fed on *L. plantarum* showed an increase in total cholesterol and in triglycerides compared with the control diet.

Ostadrahimi et al., (2015) Studied the effect of Probiotic fermented milk (Kefir) on glycemic control and lipid profile In type 2 diabetic patients. The comparison of fasting blood glucose between two groups after intervention was statistically significant. After intervention, Serum triglyceride, total cholesterol, LDL-cholesterol and HDL-cholesterol levels were not shown significant differences between and within the groups after intervention.

Abdelhady and El- Abasy, (2015) evaluated the effect of dietary supplementation of prebiotic, probiotic and their mixture on growth, biochemical parameters and immune-hematological responses of rabbits. The results showed significant increase in body weight gain, significant decrease in food conversion ratio, and significant decrease in serum total cholesterol, triglycerides and glucose when compared with control group. Also showed no significant change in ALT, AST, urea and Creatinine. Results indicated that rabbits received mixture of pre and probiotic groups recorded the highest value of daily weight gain, and recorded the lowest FCR followed by rabbits received probiotics dietary.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Chemicals

All Chemicals were bought from Murtaja Medical Corporation (MMC) Ahmed Abdul Aziz Street – ALRemal Gaza.

3.1.1.1 ELISA IGF test

Standards A-E, Sample Buffer, Phosphate buffer PP, Control Serum KS1 and KS2, Antibody Conjugate AK, Enzyme Conjugate EK, Washing Buffer (WP), Substrate (S), Stopping Solution, Sealing tape **(Alpco, 2012).**

3.1.1.2 Creatinine test

Jaffe's Alkaline Picrate Method: Picric acid – 0.04M (9.16g/l)., Sodium hydroxide – 0.75N. Sodium tungstate – 10%. 2/3 N H2SO4. 5) Creatinine standard stock–100mg%. Working standard – 3mg% **(Jagarati, 2004).**

3.1.1.3 Blood Urea Nitrogen (BUN)

Diacetyl Monoxime Method: 1) Reagent A: Dissolve 5g of ferric chloride in 20ml of water. Transfer this to a graduated cylinder and add 100 ml of ortho phosphoric acid (85%) slowly with strring. Make up the volume to 250 ml with water. Keep in brown bottle at 4 c°. 2) Reagent B: Add 200 ml conc, H2SO4 to 800 ml water in 2L flask slowly with stirring and cooling. 3) Acid Reagent: Add 0.5 ml of reagent A to 1 L of reagent B. keep in brown bottle at 4 C°. 4) Reagent C: Diacetylmonoxime 20g/L of water. Filter and keep in brown bottle at 4 Cº. 5) Reagent D : Thiosemicarbazide 5g/l of water. 6) Colour Reagent : Mix 67 ml of C with 67 ml of D and make up the volume to 1000 ml with D.H2O keep in brown bottle at 4 oC. 7) Stock urea standard : 100mg/100 nl water. 8) Working urea standard : Dilute 1 ml stock to 100ml with DH2O so conc. is 1mg/100ml % **(Jagarati, 2004).**

3.1.1.4 Blood Uric Acid

Caraway's Method of Estimation: Reagents: Sodium tungstate 10%. 2/3 N Sulphuric acid. Tungstic acid: Add 50ml of 10% sodium tungstate 50ml 2/3 N H2SO4 and a drop of phosphoric acid with mixing to 800ml water. Discard when cloudy. Store in brown bottle. Phosphotungstic acid: Stock-Dissolve 50g sodium tungstate in about 400ml of water. Add 40ml 85% phosphoric acid and reflux gently for 2 hours, cool, make volume to 500 ml. store in brown bottle. Dilute 1 to 1 for use. Na3CO3 10%. Standard uric acid solution stock-100mg%. Working uric acid solution-1mg% **(Jagarati, 2004).**

3.1.1.5 Lipid profile Test

3.1.1.5.1 Cholesterol Reagent

The components of Cholesterol high Performance System Pack Reagents (Roche Diagnostics, Indianapolis, IN) included (taken from package insert) Cholesterol Reagent (16 x 50 ml) 75 mmol/l Pipes buffer, pH 6.8,10 mmol/l Mg^{2+},0.2 mmol/l Sodium cholate, 0.15 mmol/l 4-Aminophenazone 4.2 mmol/l Phenol 0.5U/Ml Cholesterol esterase (EC3.1.1. 13;ps- eudomonassp-ecies -; 25° C) 0.15 U/ml Cholesterol oxidase(EC 1.1.3.6; E. coli; 25° C) 0.25 U/ml Peroxidase (EC 1.11.1.7; horseradish; 25° C) 1% Fatty alcohol polyglycol ether Buffer, unspecified stabilizers, unspecified preservative.

● The reagent is supplied as a solution and is ready to use. After being opened, the reagent is stable for 28 days at 2-12 °C, or 7 days at room temperature. Protect reagent from light **(NHANES, 2003-2004)**.

3.1.1.5.2 Triglyceride Reagents

The components of the Triglycerides (GPO) System Pack include (from package insert): 50 mmol/l PIPES buffer, pH 6.8, 40 mmol/l Mg^{++}

0.20 mmol/l Sodium cholate, 1.4 mmol/l ATP, 0.13 mmol/l 4-Aminophenazone 4.7 mmol/l 4-Chlorophenol 1 µmol/l Potassium hexacyanoferrate (II) 0.65% Fatty alcohol polyglycolether, 5.0 U/mLlipoprotein lipase (EC 3.1.1.13;

Pseudomonas species, 25°C) 0.19 U/ml glycerolkinase (EC 2.7.1.30; Bacillus stearotheromophilus; 25°C)2.5 U/ml glycerophosphate oxidase (EC 1.1.3.21; E. coli; 25°C) 0.10 U/ml Peroxidase (EC 1.11.1.7; horseradish; 25°C) unspecified preservative The reagent is supplied as a solution and is ready for use. When opened, the solution is stable for 14 days at 2-12° C, or 7 days at room temperature (15-25° C) **(NHANES, 2003-2004).**

3.1.1.5.3 Direct HDL-cholesterol

(a) The Direct HDL-cholesterol reagents, R1 and R2, R1 Cyclodextrin /Buffer, supplied as a solution, 0.5 mmol/l α-cyclodextrin 0.5 g/l dextran sulfate, 7.0 mg/ml magnesium sulfate ($MgSO_4$), 0.3 g/l EMSE, 10 mmol/l MOPS (3-morpholino-propane sulfonic acid) buffer, pH 7.0) 10 mmol/l MOPS (3-morpholino-propane sulfonic acid) buffer, pH 7.0) unspecified preservative (b) R2 Buffer/PEG-enzyme/4-Aminophenazone, is supplied as a lyophilized mixture and is reconstituted with diluent supplied in the reagent kit. R2 contains the following approximate concentrations after reconstitution: 1 kU/l PEG cholesterol esterase (EC 3.1.1.13; Pseudomonas species; 25° C 5.6 kU/l PEG cholesterol oxidase (EC 1.1.3.6; Pseudomonas species; 25°C 30 kU/l peroxidase (EC 1.11.1.7; horseradish; 25°C) 0.5 g/l 4-aminophenazone 10 mmol/l MOPS (3-morpholino-propanesulfonic acid) buffer, pH 7.0 Detergent and preservative **(NHANES, 2003-2004).**

3.1.1.6 Thyroid Test

IMX Ultrasensitive Htsh Ii Reagent Pack, 100 Tests (No. 4B01.20): 1 bottle (8.1) ml) anti-h TSH (mouse, monoclonal) coated micro particles in Tris buffer with protein stabilizers. Preservative: 0.1% sodium azide. 1 bottle (11 ml) anti-h TSH (goat): alkaline phosphatase conjugated in buffer with protein stabilizers. Minimum concentration: 0.1 µg/ml. Preservative: 0.1% sodium azide. 3) 1 bottle (10 ml) 4-methylumbelliferyl phosphate, 1.2 mM in AMP buffer. Preservative: 0.1% sodium azide. 1 bottle (20.2 ml) Wash Buffer containing surfactant **(NHANES, 2002).**

3.1.1.7 Liver function Test

Reagents : 1. Buffer substrate – For both enzymes, 100 mmol/l phosphate buffer and 2 mmol/l 2-oxoglutarate jwith 100 mmol/l L-asparate for AST and 200 mmol/l-DL alanine for ALT. a. for AST-add 15.7g L-asparate monosodium salt or 13.2g L- aspartic acid. b. for ALT-add 17.8g of DL-alanine. Adjust pH to 7.4 with NaOH and make up the volume to 1 Litre with distilled water. 2. 2,4 Dinitrophenylhdrazine (DNPH)-1 mmol (200 mg)/l in lmol/l HCL. 3. Sodium hydroxide solution 400 mmol (16g)/l. 4. Pyruvate solution – 2 mmol/l (22mg of sodium pyruvate in 100 ml of distilled water **(Jagarati, 2004).**

3.1.2 Equipments

Lithium heparinized blood collection tubes.1cc or 3 cc syringes. Reference analyzer (as determined by facility). Centrifuge (if reference analyzer does not accept whole blood). Blood Sample Tube Mixer/Rotator Microtiter plate. Distilled water. Micropipettes and multichannel pipettes with disposable plastic tips. Vortex-mixer. Micro titer plate Shaker (350 rpm). Microtiter plate washer (recommended). Microtiter plate reader ("ELISA-Reader") with filter for 450 and 590 nm. Polyethylene PE/Polypropylene PP tubes.500 ul positive displacement pipettes (SMI, Inc.). 250 ul positive displacement pipettes (SMI, Inc.). 50 ul pipettes, (Absoluter). 1.4 mL micro centrifuge tubes Wheaton Step-PetteStop clock. Test tubes. Water bath at 37 °C Pipettes (0.2, 1 and 10 ml).Spectrophotometer and cuvettes .Instrument Beckman Synchron LX20. Beckman Synchron CX Micro Sample Tube (Part #448774) , S/P Plastic Transfer Pipet (Cat. #P5214-10),S/P Brand Accutube Flange Caps (Cat. #T1226-37) **(NHANES, 2002-2005).**

3.1.3 Kefir grains & samples

kefir grains were obtained from Mrs. Al Najar which she that live at Nusierat Camp. The method of making Kefir is occurred by directly adding Kefir grains to the pasteurized milk (Almazraa) generally 50g /l. After a period of fermentation, 18-24 hours at room temperature, the grains separated from the milk by filtering with a sieve for using in the next inoculation **(Semih and**

Cagindi, 2003). Then the sieved milk was diluted by water to (10%) and (20%) respectively. Some actual nutrient contents of Kefir were examined in Ministry of National Economy Labs.

3.1.4 Animals

Domesticated Rabbits which aged 35-40 days, sexual mixed and weighed average 670 ± 35 g were obtained from the local market.

3.2 Method

3.2.1 Study design.

The present study design is a case-control.

3.2.2 Study population

Study populations are consisted of two rabbit groups that drank 10% & 20% Kefir milk mixed with water (cases) and compared with the rabbits that drank water only. Each group were contained 8 rabbits. Cases and control matched each other's in age, initial weight, food intake, and all other environmental conditions.

3.2.3 Sampling, and sample size

3.2.3.1 study samples

The study sample included 24 rabbits. They divided into three groups, each group have 8 rabbits. First group was a control. The case groups are the rabbits that were fed a kefir mixed with water, 10% and 20%, respectively.

3.2.3.2 Blood sample collection

Two rabbits from each group were selected randomly for slaughtering. Blood sample were collected from cases groups and control. Then they were left for half an hour to coagulate at room temperature and the plasma were removed for biochemical analysis. (TC), (TG), (HDL)- cholesterol levels were measured in serum samples by using enzymatic method kits (Roche Diagnostics). HDL, LDL, were estimated by precipitation with sodium phosphor tungstate-magnesium chloride and sodium dodecyle sulphate reagents. IGF by **ELISA.** ALT and AST were assayed by **colorimetric**

methods Modified Reitman & Frankel Method. Creatinine levels was measured by **(Creatinine AMR Testing Procedure. ITC,2005).** Blood Urea Nitrogen & Blood Uric Acid was carried by **(Beckman Synchr- on LX20).** Total thyroxin was runned by **ELISA** . Blood glucose levels were measured by the glucose-oxidase **method using an Accu- chek blood glucose meter.**

3.2.4 Feeding procedures

Each experimental group consist of 8 rabbits. Average wt ± (SD) 670g ± 35.30 kept in a normal atmosphere**,** temperature. Animals cases were fed kefir for 6 weeks. Daily intake of kefir was 10%, 20%, respectively. Kefir was applied in drinking water. Feed and water will offered *adlibitum*. All groups of rabbits (including control rabbits) received simultaneously a commercial balanced diet rabbits fodder (Anber). The fodder components were analyzed by Islamic University Food Analysis Labs. The chemical analysis of fodder diet was compared with ingredients percentage shown on the commercial label.

3.2.5 Feed conversion and growth determination.

All Rabbits were individually weighed firstly and then at weekly intervals until the end of the experiment. Feed consumption of each experimental unit were recorded daily and feed conversion ratio were calculated by dividing the average daily feed intake (g), on Average daily growth rate (g), for each study group. The study included the total body weight gain, total feed intake, average daily growth rate and feed conversion ratio. The organs from slaughtering rabbits for each group weighed individually.

3.2.6 Result analysis

All obtained data were analyzed by ANOVA using SPSS (V20) system. Difference between variables will considered statistically significant if p value < 0.05.

Standard error mean: SEM is calculated as the standard deviation divided by the square root of the sample size.

Chapter 4

Results

4.1 Chemical composition of the commercial fodder diet

Table 4.1 shows the chemical composition of the commercial fodder rabbits Anbar (FRA) diet. The chemical analysis of FRA diet was compared with ingredients percentage shown on the commercial label.

Table 4.1: Chemical composition of commercial fodder rabbits (Anbar)

Ingredients	*labeled	**tested
Total protein	17.00	19.30
Water	13.00	10.30
Total fats	3.50	8.50
Fibers	10.50	***N
Ash	7.50	7.65
Calcium	0.80	0.82
Phosphorous	0.60	***N
Salt	0.65	0.51
Magnesium	0.04(mg/kg)	***N

*According to the commercial label

**According to results of Islamic University Food Analysis Labs.

*** N non-tested.

As shown in the table 4.1, there were slight increase in total protein and more than 100% increase in total fat. Also there were slight decreases in water and Salt. Ash was very slightly increased compare to the commercial label.

4.2 Chemical compositions of the kefir

Table 4.2 shows the chemical composition of the kefir milk drink which used as partial feeding of the growing rabbits, as analyzed by Ministry of National Economy Labs, the kefir milk drink contains 2.98 %, 3.00 %, 3.61 % proteins, fats and carbohydrates, respectively.

Table 4. 2 : Chemical compositions of the kefir

Ingredients	*%
Water	89.81
Protein	2.98
Fat	3.00
Lactose	3.61
Energy	53.36 kcal
Calcium mg/100gm	200.00

***According to results of Ministry of National Economy Labs**

4.3 Growth rate of the rabbits

In table 4.3, the initial body weight were the same. After four weeks of starting experiment, a significant decrease in body weight of T1 compared to control or T2 was observed accordingly, this significant decrease in body weight was reflected in growth rate ($P < 0.05$). In contrast, no significant difference was observed between T2 & Control group with respect to body weight or growth rate.

Table 4. 3 : Average (± SEM) of final body weights of the rabbits after 4 weeks from the experiment.

Average body weight	Control (C)	(T1)	(T2)
Initial, g	a 670.00 ± 27.00	a 670.00 ± 40.90	a 670.00 ± 38.20
Final, g	**a** 1478.75 ± 45.72.	**b** 1419.00 ± 56.00	**a** 1499.00 ± 49.39
Total, g	**a** 794.29 ± 39.74	**b** 768.00 ± 29.35	**a** 800.67 ± 23.00
Growth rate, g/day	**a** 28.36 ± 1.42	**b** 27.43 ± 1.05	**a** 28.59 ± 0.82

Means with different superscripts in the same row differ significantly (p <0.05)

C: control (T1): Trial one of 10% Kefir (T2): Trial two of 20% Kefir

Table 4.4 shows average (± SEM) of change of body weight and growth rate of the rabbits after 6 weeks of growth. A significant decrease body weight and growth rate of T1 compared to the control group or T2 was observed after

6 weeks of growth. There were no statistically differences between control and T2. As the same result after 4 weeks.

Table 4.4 : Average (± SEM) of final body weights of the rabbits after 6 weeks from the experiment.

Average body weight	(C)	(T1)	(T2)
Initial, g	**a** 670.00 ± 27.00	**a** 670.00 ± 40.90	**a** 670.00 ± 38.20
Final, g	**a** 1816.43 ± 64.40	**b** 1721.25 ± 84.54	**a** 1838.83 ± 76.48
Total, g	**a** 1132.14 ± 56.52	**b** 1094.50 ± 52.72	**a** 1198.50 ± 37.90
Growth rate, g/day	**a** 26.33 ± 1.30	**b** 25.45 ± 1.23	**a** 26.55 ± 1.51

Means with different superscripts in the same row differ significantly (p <0.05).

C: control (T1): Trial one of 10% Kefir (T2): Trial two of 20% Kefir

4.4 Feed intake and feed conversion ratio of the rabbits.

The effects of Kefir on average daily FI and FCR are summarized in Tables 4.5 and 4.6. Kefir supplementation seems decreasing the average daily FI and FCR. In tables 4.5 & 4.6 the lowest total feed consumption and FCR values were observed in Trial (T2). The initial feed intakes in first week, showed no statistically differences between the different groups, but at the end of experiments significant decrease was observed in the weight of fodder, and average daily intake among groups. Cases consumed little feeds than control groups. As kefir % increased, the cost of feeding of rabbits decreased.

Table 4.5 The average (± SEM) feed intake and feed conversion ratio of growing rabbits fed kefir differently after 4 weeks from the experiment.

	Dietary group		
Parameters	**C**	**T1**	**T2**
Initial feed intake g	**a** 58.59 ± 2.02	**a** 56.32±1.63	**a** 55.47±1.41
Final feed intake g	**a** 100.47±2.90	**b** 84.28±2.99	**c** 71.81±.11
Average daily feed Intake (FI) g	**a** 77.64 ± 3.56	**b** 69.07± 2.34	**c** 65.00 ± 1.53
Feed conversion ratio (FCR)	**a** 2.66± 0.05	**b** 2.43 ± 0.03	**c** 2.23 ± 0.06

Means with different superscripts in the same row differ significantly (p <0.05)

C: control (T1): Trial one of 10% Kefir (T2): Trial two of 20% Kefir

In table 4.6 similar results was observed after 6 weeks of the experiment. Initial feed intake had no difference between Trial (T1) and Trial (T2) and both of them showed non-significant difference with the control group. The final FI, average daily FI, FCR were had a statistically significant decrease between cases and control. This decrease was more pronounced as Kefir concentration increased see table 4.5 (see T2).

Table 4.6 The average (± SEM) feed intake and feed conversion ratio of growing rabbits fed kefir differently after 6 weeks from the experiment.

	Dietary groups		
Parameters	**C**	**T1**	**T2**
Initial feed intake ,g	**a** 58.59 ± 2.02	a 56.32 ±1.63	**a** 55.47 ±1.41
Final feed intake ,g	**a** 105.99 ±2.66	**b** 100.00±1.89	**c** 84.13±.2.93
Average daily Intake ,g	**a** 87.35 ± 3.14	b 79.75±2.80	**c** 72.73± 2.04
Feed conversion	**a** 3.54 ± 0.17	**b** 3.16 ± 0.15	**c** 2.87 ± 0.17

Means with different superscripts in the same row differ significantly (p <0.05)

C: control (T1): Trial one of 10% Kefir (T2): Trial two of 20% Kefir

4.5 Organs and carcass weights.

Table 4.7 shows the results of some average (± SEM) organs weights of the experimental groups.

Table 4.7 Effect of partially kefir intake on some average (±SEM) organs weight, body fat and carcass weight of the growing rabbits.

Average organs and carcass weight, g	**C**	**T1**	**T2**
Skin	**a** 182.00 ± 7.50	**a** 187.00 ± 7.50	**b** 170.00 ± 10.00
Head	**b** 157.00 ± 2.50	**a** 170.00 ± 5.00	**a** 177.00 ± 7.50
Legs	**a** 60.00 ±.5.00	**a** 62.00 ±2.50	**a** 60 ± 5.00
Viscera	**b** 250.00 ±5.00	**c** 242.00 ±2.50	**a** 262.50 ± 2.50
Liver	**a** 60.00 ± 5.00	**c** 50.00 ± 5.00	**b** 55.00 ± 2.00
Kidney, Spleen and lungs	**a** 35.00 ± 5.00	**a** 32.5 ± 2.5	**b** 22.5 ± 2.50
Carcass	**b** 940.00 ± 10.00	**b** 937.00 ±2.50	**a** 952.00 ±2.50
Internal body fat tissue	**a** 22.00 ± 2.50	**b** 12.50 ± 0.50	**c** 9.00 ± 1.00

Means with different superscripts in the same raw differ significantly(p<0.05)

C: control (T1): Trial one of 10% Kefir (T2): Trial two of 20% Kefir

As shown in table 4.7,there were significant decreases in weights of skin, kidney, spleen, lungs, liver and internal body fats as Kefir concentration in water increased. In contrast, as Kefir concentration increased, Viscera and carcass weights increased. There was significant decrease in T2 compared to C & T1, but there was not any significant difference between C & T1. The head weight showed significant increase in T1 & T2. The average of leg weight showed non-statically significant among groups (P= 0.898). The lowest average weight of Viscera was observed in T1. The liver weight was decrease in the cases groups compared to control. The weight of Kidney, Spleen and lungs was significantly decreased in T2 compared to C & T1. The highest

average values of carcass was observed in T2. The Internal body fat tissue weights was decreasing as Kefir increasing, (P= 0.019).

4.6 Biochemical parameters of rabbit serum.

Table 4.8 summarized some biochemical parameters of rabbits serum. there was a significant decrease in FBS, IGF1, LDL, FT4 and uric acid among study population as concentration of Kefir increased. In contrast, there were a significant increases in total cholesterol, TG, ALT, AST and Creatinine among study population as concentration of Kefir increased.

Fast blood sugar was decrease in cases, as the kefir increases, but there was no statically difference between T1 & T2.

The IGF showed the lowest value in T2 (8.85±.6.3 Ng/ml), it decrease with kefir increase. But there was no statically difference between C & T1. There was a significant decrease between T2 & C and between T2 & T1.

The total cholesterol and Triglyceride was increased in cases, whatever Kefir treatment increase. But there was no significant differences between C & T1, There was significant differences between T2 & C and between T2 & T1. LDL tend to decrease in response to Kefir milk increase. There was statically significant between T2 & C and between T2 & T1. In our study HDL-c wasn't had any difference between three groups (P >0.05).

About kidney function parameters, Urea and Uric Acid was showed non-significant difference among all groups. Uric Acid tend to reduce in response to increasing kefir, the lowest value was in T2. Creatinine level was highest in T2 (1.65 ± .05), but there was not any significant differences between C and T1 and There was significant differences between T2 with C and T2 with T1

The liver enzymes AST and ALT were higher in cases than control. AST is The highest value in T1. ALT is The highest value in T2. There was a difference in AST between groups but non-significant. In ALT there was not

any difference between C and T1, and there was significant differences between C & T2 and T1 & T2.

The FT4 decrease in cases, there was significant decrease between C&T1 and C&T2.

Table 4.8 Average (± SEM) Biochemical parameters of rabbit serum

parameters	C	T1	T2	Unit
FBS	a 102.00 ± 5.50	b 88.50± 3.50	b 89.00± 2.00	**Mg/dl**
IGF1	a 38.85± 16.20	a 43.20 ±22.30	b 8.85.00±.6.3**0**	**Ng/ml**
Total cholesterol	b 43.00 ± 1.0**0**	b 44.50± .5.00	a 56.00 ± 2.00	**Mg/dl**
Triglyceride	b 121.00± 9.00	b 118.00 ± 14**.00**	a 136.00 ± 3.50	**Mg/dl**
LDL	a 33.00± 1.00	a 32.50± 5.5**0**	b 22.50± 9.50	**Mg/dl**
HDL	a 52.00± 4.00	a 54.00± 2.00	a 51.50± 12.50	**Mg/dl**
Urea	a 22.50± 0.5	a 23.00± 1.00	a 24.50± 0.50	**Mg/dl**
Uric Acid	a 6.55± 0.65	a 5.35± 0.15	a 5.00± 0.30	**Mg/dl**
Creatinine	b 1.25± 0.05	b 1.25±0.05	a 1.65± 0.05	**Mg/dl**
AST	c 23.5± 10.50	a 40± 0.00	b 35.5±0 .50	**u/l**
ALT	b 34.5± .5.0	b 36.5± 2.5	a 45± 15.0	**u/l**
F T4	a 1.40± 0.40	b 1.05± 0.15	b 1.10± 0 .20	**Ng/dl**

Means with different superscripts in the same raw differ significantly (p<0.05)

C: control (T1): Trial one of 10% Kefir (T2): Trial two of 20% Kefir

Chapter 5

Discussion

5.1 Characteristic of the Study population

The present study is a case control investigation, comprised two cases groups, and one control group. The experiments carried out at June and July 24 mixed sex local rabbits that aged 35 – 40 day were bought from local market. They were left 12 days for adaptation before the beginning of the experiment. During this period, they received tetracycline and vitamins with water. The 24 rabbits divided into the three groups, 8 rabbits for each group. Control one received water only and two cases that drinking kefir milk with water (10%- 20%) respectively. The mean weights of controls and cases were (670±27.00), (670±40.90), and (670±38.20) respectively. They were fed fodder rabbits Anber (FRA) freely, and were lived in same condition.

5.2 Kefir preparation and contents

The Kefir milk prepared by adding the whole milk to Kefir grains, and left it at room temperature for fermentation followed by filtration. There was a difference between tested sample (table 4.2) and composition in table 1.1 especially Ca value. The differences between chemical composition of kefir might refers to the different types of milk (various species, various levels of fat) and different production methods **(Torshizi et al., 2010)**.

5.3 Fodder analysis (Anber)

The analysis of rabbits fodder's diet was compared with ingredients percentage shown on the commercial label. There were a clear increase in the actual concentrations of the total crude protein, and total fat. In contrast, there was a clear decrease in the actual concentration of salt and water. There was a slight increase in ash. Previously, similar findings were reported by **Zabut et al, 2007**. One can conclude labeled composition differs from actual compositions, that affect the anabolism processes on the body.

5.4 Body weight gain of the rabbits

People raise rabbits for meat production, because they requires a way less food and water to produce meat greater than other animals. For example, if a cow and a rabbit were fed the same amount of food and water, a rabbit will produce meat six times a cow. Their meat is lower in cholesterol, also it has the highest protein ratio. Domestic raised rabbits are white meat, tender, juicy and mild in flavor. Rabbit meat has the least number of calories per pound and has only 8 percent bone. It is not only lean and nutritious, but it's also tasty **(Wolf, 2014).**

The final weight after 4 weeks was the highest in T2 and the lowest in T1. After 6 weeks the same results was obtained, the highest in T2 and the lowest in T1 compared to control. There were non significant differences in terms of body weight gain. Similarly results were obtained by **Sahin and Yardimci, (2009), Ataşoğlu et al., (2010), Erkan et al., (2012), Reza et al., (2013), Salaj et al., (2013), and Piccolo et al., (2014).** In contrast, other studies reported that live weights of the study groups were significantly increased compared to that of the control group **(Cenesiz et al., 2008)**,. Moreover the Performance of broilers and rabbits in terms of body weight gain, improved when probiotic was provided via drinking water, compared to the control and feed groups **(Torshizi et al., 2010)**. **(Abdelhady and El-Abasy, 2015)**. Our finding showed The final body weight gain of the rabbits were not improved when kefir was provided via drinking water, compared to the control. May be this refer to concentration and type of microorganism in probiotic food.

5.5 Feed conversion of the rabbits

The present findings showed that there was significant decreases in FI and FCR as percentage of kefir concentration increases compared to the control one. In other words, the cost of feeding rabbits decreased as Kefir concentration in water increased. This result reported by **(Reza et al., 2013), (Abdelhady and El-Abasy, 2015)**. As they reported beneficial effect of probiotic supplementation to broiler and rabbit diet in terms of increased feed conversion through a natural physiological way and improving digestion by balancing the resident gut microflora as they can improve the integrity of the

intestinal mucosal barrier, digestive and immune functions of intestine. Improvement in digestion and absorption of intestine of nutrient transportation systems leads to immune resistance and productivity. In contrast, another studies reported that FI & FCR values were similar in all experimental groups during whole the trial period **(Sahin and Yardimci, 2009)**.

5.6 Body Organs Weight of the gain of the rabbits

The present findings showed that there was significant decreases in weights of skin, Kidney, Spleen, lungs, liver, Internal body fat tissue as percentage of kefir concentration increased to 20%. In contrast, there were significant increases in weights of Carcass, Viscera as percentage of kefir concentration increased. But there were no significant change between all groups. These finding were consistent with **Urdaneta et al., (2007) And (Sahin and Yardimci, 2009).** The finding that weight of Caracas increased and weights of organs decreased was very significant on human feeding. On the other hand, a gradual decrease was seen in Internal body fat tissue based on the increased kefir rates **(Urdaneta et al., 2007), (Sahin and Yardimci, 2009).** Increasing in Weights of Carcass of T3 may be refer to high protin diet in Kefir and fodder.

5.6 Biochemical blood profile

We found in our present study that, significant decreases in FBS, IGF1, LDL, FT4, as percentage of kefir concentration increased to 20%. These finding consistent with low growth rate daily FI and FCR in cases compared to the control after 4 weeks and 6 weeks of growth. In contrast, the findings showed increases in TC, TG, ALT, AST which reflect higher liver activity as kefir concentration increased. Effect of kefir on kidney function was not clear and required further investigations, because of increasing level of Creatinine. Although Creatinine concentration increased, urea concentration were not affected. Uric acid were decreased slightly with increased kefir diet in response to control, that is lowering the probability of causing gout.

FBG was reduced in response to kefir diet increase. In some studies was reported that kefir reduced glycemic index in diabetic patient **(Urdaneta et al., 2007), (Ostadrahimi et al,. 2015) (Judiono et al 2014) and (Abdelhady and El-Abasy, 2015)**.

The IGF1 is the lowest value in T2 (8.85±6.30), it is very low comparing to control and T1. The T2 group has a normal growth , normal value and a normal weight. This reason may be refer to exist IGF in binding form (IGFBP3). If the IGF1 is low. that is not a parameter for GH deficiency. Neither IGF-I nor IGFBP-3 alone is a marker for growth hormone insufficiently. In addition, they cannot be used as an effective screening test in combination **(Mitchell et al., 1999)**. No previous study targeted IGF1 in treatment with Kefir, however this parameter need more investigation . If a decrease in IGF-1 is suspected to be due to a more general decrease in pituitary function. Also may be seen with nutritional deficiencies, chronic kidney or liver disease. stressful **(Alpco, 2012 and Marcello et al., 2013).** High blood levels of IGF-1 are associated with premature aging and diseases of aging such as diabetes and cancer. IGF-1 shortens life by increasing cell DNA genetic damage, and causes cancer by blocking apoptosis that causes cancer cells to kill themselves before they destroy their host **(Wafer, 2011).**

The highest values of total cholesterol and Triglyceride in group T2 that received high dose of kefir. These finding may be because of usage whole milk. **Farnworth, (2008)** said that one study reported that yogurt increase the total cholesterol.. The LDL (bad lipoprotein) reduced, however increasing kefir percentage. The reduction of LDL-C must be of prime concern in the prevention of Cardio Vascular Disease. High density lipoprotein (good lipoprotein) values were approximately similar in all groups. In our study HDL-c wasn't any difference between three groups (p = 0.971**).** There was non-significant among groups. The previous findings are in agreement with that obtained by **Marie-Pierre et al. (2002) an**d **Ostadrahimi et al. (2015).** Other studies reported that Kefir tended towards a lowering of serum triacylglycerol and total cholesterol concentrations **(Je-Ruei Liu et al., 2006)**. Total cholesterol serum and total lipid levels were significantly reduced compared to that of in control group in response to kefir treatment **(Cenesiz**

et al., 2008). Michel et al., (2013) reported total cholesterol, HDL-c, LDL-c and triacylglycerols in rats fed cholesterol-rich diet, fermented kefir reduced significantly the levels of VLDL, LDL-c and triacylglycerols, in addition to having increased HDL-c.

Urea level was not affected by Kefir diet. Creatinine was slightly increased in T2 compared to control and T1. There were no significant differences between three groups. **Urdaneta et al., (2007)** reported the same result about uric acid**.** That is mean Kefir milk does not affect the kidney function. Uric acid level in all groups were higher than normal value. This result may be refer to high purine amino acid in fodder diet, however uric acid is the end product of complete catabolism of purines (**Barbara et al., 2002**) or may be Thermally stressful **(Michaelson and Lin, 2013), (Al-Jubury, 2011).**

AST and ALT increased in experimental group (T1, T2), but there was no statistically significant between groups. **(Sahin and Yardimci, 2009 and Cenesiz et al., 2008)** were showed that The effect of Kefir on serum AST and ALT activity in the groups did not result in any changes.

In the present study our finding that free T4 was decrease whenever kefir treatment increase. the highest value was in control. T1 and T2 were slightly the same value. However, according to my knowledge there is not any previous study targeted free T4 in treatment with Kefir. The present study is the first to assess free T4 with kefir treatment. **Dale,(2012)** reported that Drinking kefir daily will give Hypothyroidism patient's system the rebalancing it needs to absorb Synthroid correctly. Good bacteria support conversion up to 20 % of thyroxin (T4) to triodothyreonine (T3) in the intestine. Having not enough bacteria makes less active T3 hormone available causing hypothyroidism symptoms **(outsmart disease, 2015).**

Chapter 6

Conclusion and Recommendations

6.1: Conclusion

- There were significant decreases in growth performances in case T1 rabbits that treated with 10% Kefir. That was very clear after 6 weeks of growth, compared to those rabbits fed 20% Kefir and only FRA.
- There were significant decreases in average daily feed intake and feed conversion ratio among cases compared to the control. Cases, thus cost with as reacts to feed intake less than control.
- There were significant decreases in skin weight, kidneys, spleen, lungs, liver and internal body fat as Kefir concentration increased.
- In contrast There were significant increases in Caracas and viscera weight as Kefir concentration increased.
- As Kefir percentage increased, There were significant decreases in FBS, IGF1, LDL, FT4,
- As Kefir percentage increased, There were significant increases in Total cholesterol, TG, AST, ALT.

6.2: Recommendations

- Further studies are needed for using larger rabbits samples and other different kefir concentration.
- Effect of kefir on other biochemical blood profiles such as Leptin, adiponectin and thyroid hormone also recommended.
- I hope usage of kefir as a natural food to reduce the weight and internal body fats .

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