EFFECT OF DIETARY JERUSALEM ARTICHOKE AND CHICORY SUPPLEMENTATION ON PERFORMANCE, SERUM CHOLESTEROL LEVEL AND GUT MICROFLORA OF JAPANESE QUAIL (*COUTRNIX JAPONICA*)

Omed Ahmed ISMAEL

MASTER THESIS

ANIMAL SCIENCE Department

Supervisor: Associate Prof. Dr. Bünyamin SÖGÜT

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PREFACE

First and above all, I praise God, the almighty for providing me this opportunity and granting me the capability to proceed successfully. This thesis appears in its current form due to the assistance and guidance of several people.

I am forever grateful to my parents, Ahmed (father) and Mryam (mother), and my sisters and brothers, for their love and steadfast support, and for believing in me and melding me into the individual I have become.

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Omed Ahmed ISMAEL

Bingol 2017

DEDICATION

To my loving parents (Mother & Father), who is my greatest inspiration, always pick me up on time and encourage me to go on every adventure, Specially this study.

To my amazing wife (Tavga) who perfectly care about me and helpful at finishing this work.

To all my sisters and brother who my biggest support and advisers in a right ways.

CONTENTS

LIST OF ABBRIVATION

ANOVA	:	Analysis of variance
AGP	:	Antibiotic growth promoter
BGW	:	Body weight gain
CFU	:	Colony forming unit
D	:	Day
E. coli	:	Escherichia coli
FCR	:	Feed conversion ratio
FI	:	Feed intake
FOS	:	Fructooligosaccharide
GI	:	Gastrointestinal
JA	:	Jerusalem artichoke
LAB	:	Lactic acid bacteria
LBW	:	Live body weight
MOS	:	Mannanoligosccharides
MRS	:	Man, Rogosa and Sharp
PBS	:	Phosphate buffers solution

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BİR PREBİYOTİK KAYNAĞI OLARAK RASYONA DEĞİŞİK SEVİYEDE YERELMASI VE HİNDİBABA EKLENMESİNİN BILDIRCINLARIN BÜYÜME-GELİŞME, KARKAS ÖZELLİKLERİ, KOLESTOL VE BARSAK FLORASINA ETKİSİ

ÖZET

Bu çalışma, bir prebiyotik kaynağı olarak rasyona %1 ve %0,5 seviyesinde yer elması ve hindibaba bitkisinin bıldırcınların büyüme performansı, karkas özellikleri,kolestrol içeriği ve barsak florası üzerine etkisini belirlemek içinyapılmıştır. Toplam 225 adet günlük bıldırcın civcivleri şansa bağlı olarak 3 tekerrülü 5 muamele grubuna şansa bağlı olarak dağıtılmıştır. Deneme, control (T1, katkı maddesi içermeyen), % 0,5 Yerelması elması içeren (T2), %1 Yerelması içeren(T3), %0,5 Hindibaba içeren (T4) ve%1 Hindibaba içeren (T5)gruplarından oluşmuştur. Hayvanların yemleme ve sulamaları 6 haftalık deneme süresince *adlibitum* olarak yapılmıştır. Bıldırcın rasyonuna prebiyotik kaynağı olarak Yerelması ve Hindibaba eklenmesi bıldırcınları 6 hafta sonu canlı ağırlık, canlı ağırlık kazancı, yem tüketimi, yemden yararlanma oranı ve karkas kalitesi üzerine istatistiki olarak herhangi bir etkisinin olmadığını gözlenmiştir (P>0,05). Ayrıca, bakteri sayıları ve kolesterol içeriği her iki prebiyotikten de etkilenmemiştir. Bununla birlikte, göğüs eti rengi, besleyici prebiyotiklerin kullanımından etkilenmiştir. T3 ve T4 gruplarında kaydedilen et rengien iyi sonucu vermiştir. Dişi bıldırcınların etlerindeki parlaklık (L *) değeri, %1Yerelması ve %0,5 Hindibaba gruplarında, %0,5 Yerelması, %1 Hindibaba ve control grubuna göre daha yüksek bulunmuştur (P<0,05). Genel olarak, her iki prebiyotik tipinin farklı düzeylerde bıldırcın rasyonuna katılmasının üretim performansını olumlu etkilemediği, buna karşılık et rengini iyileştirebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Prebiotik, yer elması, hindibaba, quail, bağırsak florası, et rengi, kolesterol.

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ABSTRACT

In this study Jerusalem artichoke tubers and Chicory powder as a source of prebiotics at 0.5% and 1% levels were supplemented to determine the growth performance, carcass characteristics and cholesterol content in quail chicks. An experiment of 42 days was conducted with a flock of 225 one-day old chicks, were used in a completely randomized design (CRD with 5 treatments and 3 replicates). Treatments were control no additive (T1), T2 containing 0.5% Jerusalem artichoke, T3 containing 1% Jerusalem artichoke, T4 containing 0.5% Chicory and T5 containing 1% of Chicory. All chicks had free access to feed and water ad libitum during the 6-wk experiment. The results showed the both type of dietary prebiotic supplementations had no effect on the live body weight, weight gain, feed intake, feed conversion ratio and carcass quality at the end of the experiment. Also, the bacterial counts and cholesterol content had no affected by both type of prebiotics. While, the breast meat colour affected by using the dietary prebiotics. The best result of meat colour recorded for the T3 and T4. The colour lightness (L* value) of the female was increased in 1% of Jerusalem artichoke and 0.5% of Chicory supplementations compared with 0.5% Jerusalem artichoke, 1% of Chicory and control group. Overall, it was concluded that different level (0.5 and 1%) of both type of prebiotics cannot positively affect production performance, while, can improve the colour of meat.

Keywords: Prebiotics, jerusalem artichoke, chicory, quail, gut flora, meat colour, cholesterol.

1. INTRODUCTION

Enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality, and the associated contamination of poultry products for human consumption (human food safety). With increasing concerns about antibiotic resistance, the ban on sub therapeutic antibiotic usage in Europe and the potential for a ban in the United States, there is increasing interest in finding alternatives to antibiotics for poultry production.

Prebiotics are several approaches that have potential to reduce enteric disease in poultry and subsequent contamination of poultry products. Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid 1995).

Prebiotics such as Mannanoligosaccharids (Flemming et al. 2004), Fructooligosaccharides (Verdonk and Leeuwen 2004) and inulin (Roberfroid 2007) (Sofia and Gibson 2007) (Rehman et al. 2008) enhance the growth of intestinal bacteria and may affect the intestinal histology.

This experiment was conducted using two very common plants as a prebiotic (Jerusalem artichoke and Chicory), which they are two plants rich in prebiotics in their underground parts.

Inulin derived from some kind of plants such as Jerusalem artichoke, chicory, garlic, onion, asparagus; leak; banana, dandelion (Van Loo et al. 1995). Jerusalem artichoke and chicory are natural sources of inulin those are rich in inulin (Kaur and Gupta 2002)(Stolzenburg 2005). Additions of inulin from chicory was found to affect positively

on performance in monogastric animals (chicken, pig, rabbit, and rat). However, in poultry, very few reports have focused on the effect of inulin from Jerusalem artichoke and Chicory on the gut microflora of the poultry gastrointestinal tract at the present time.

The aims of this study:

The overall aim of this study was to investigate the effect of two different types of prebiotic supplementations, Jerusalem artichoke tuber (Helianthus tuberosus) and Chicory roots (Cichorium intybus) on production performance and the gut microflora of Japanese quail. The specific objectives of this study were addressed by the following:

- To investigate the effects of prebiotics on growth performance on Japanese quail.
- To investigate the effects of prebiotics on intestinal microflora by culture based technique.
- To investigate the effects of prebiotics on serum cholesterol content.
- To investigate the effects of prebiotics on meat quality via the study of colors of meat.

2. LITERATURE REVIEW

2.1. The concept of prebiotic

Prebiotics are defined as ‘a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid 1995). In other words, prebiotics are provided as a substrate for beneficial microorganisms in the gastrointestinal tract. Large amounts of beneficial bacteria are capable of consuming and digesting these types of carbohydrate sources for energy, where consequently cause increased activity of beneficial bacteria (Hillman 2001).

There are some characteristics of prebiotics as an effective source to promote beneficial impact on poultry production and their health status.

According to Gibson et al. (2004), to be classed as a prebiotic, a dietary substrate is supposed to follow three criteria:

1. The substrate must not be digested by endogenous enzyme in the upper gastrointestinal tract.

2. It must be fermented specifically by beneficial commensal bacteria in large intestine.

3. Effects on the microbiota must be selective and associated with host health promotion.

2.2. The Action of Prebiotic

Prebiotics are non-digestible in the upper part of intestinal tract. Prebiotic that has a beneficial effect through their selective metabolism in the intestinal tract (Gibson et al. 2004). The ability of a lactic acid bacteria strains to survive in the gastrointestinal tract may be promoted by oligosaccharides facilitating the metabolism and growth of lactic acid bacteria in the lumen (Salminen et al. 1998a). Dietary fibre, mainly oligosaccharides and polysaccharides fermented in the colon may act as prebiotics (Ziemer and Gibson 1998)(Fooks et al. 1999). The importance of prebiotics as enhance of the growth and performance of probiotic bacteria has been documented in humans (Fooks et al. 1999) (Crittenden et al. 2002).

Feeding prebiotic (fructans) from chicory to broilers may improve weight gain, feed conversion and carcass weight. Feeding chicory fructans may also have systemic effects like a decrease in serum cholesterol levels and deposit of fat tissue (Yusrizal and Chen 2003). The selective interaction between prebiotics and the intestinal flora result in increased intestinal colonization resistance. This was demonstrated by Kleessen et al. (2003) who found lower numbers of total aerobes, Enterobacteriaceae, and C. perfringens counts by supplement of fructan-rich from Jerusalem artichoke and increased significantly ($P < 0.01$) B. bacteriovorus counts in caecum, as well as reduced levels of endotoxins in the blood compared with control birds. Therefore, Jerusalem artichokes stimulate growth of broiler chickens and protect them against endotoxins and potential caecum pathogens. Mannanoligosaccharids (MOS) is another type of prebiotic that acts by binding and removing pathogens from the intestinal tract and stimulating the immune system (Patterson and Burkholder 2003). Bacteria attach to the intestinal cells of the host with type 1 fimbriae and this attachment enables the bacteria to cause disease in the host

(Figure 2.1). Mannose, the main component of Mannanoligosaccharides, is a unique sugar, which also contains receptors for type 1 fimbriae. Mannanoligosaccharides functions as a competitive binding site to which the bacteria bind, after which they are carried out of the gut instead of binding to the intestine. Salmonella typhimurium colonisation of the intestine was decreased when 2.5% mannose was applied in the drinking water of broilers (Giggs and Jacob 2005). Type 1 fimbriae are adhesion organelles expressed by many Gram-negative bacteria, and presence this kind of bacteria greatly enhances the bacteria's ability to attach to the host and cause disease (Connell et al. 1996).

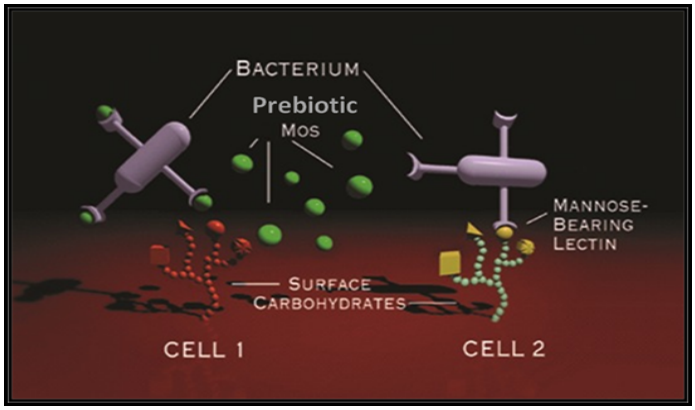

Figure 2.1. Blocking bacterial attachment and thus inhibiting host colonization by Mannanoligosaccharides as prebiotic (Wysong 2003)

2.3. Jerusalem Artichoke And Chicory as Prebiotic

2.3.1. General Characteristics of Jerusalem Artichoke And Chicory

The common name for this plant in the world is Jerusalem artichoke (Helianthus tuberosus). The Jerusalem artichoke also called Sunroot, Sunchoke or earth apple is a species of sunflower native to eastern North America.

Figure 2.2. The plant (Top) and the tubers (Bottom) of Jerusalem artichoke

Asterace as family (Compositae) that is grown as an annual crop. The tops die in the early winter and the tubers are harvested at which time in the winter. The plant grows under different climatic conditions and shows a good frost and drought tolerance as well as resistance to pets and diseases (Slimestad et al. 2010). Jerusalem artichokes store carbohydrates in the form of inulin instead of starch. Inulin is a fructooligosaccharide, which has a range of healthy characteristics. Inulin can be regarded as a dietary fibre, a straight chain of fructan and it is not digested by enzymes in the digestive system by human. Inulin can be used as a bulking agent in foods when sugar is replaced with an artificial sweetener. The volume previously occupied by sugar is replaced by the low-calorie inulin, allowing the total caloric content of the processed product to be greatly

reduced. With little reformulation, inulin, though not sweet, functions similar to sugar, such as, browning reactions, aroma synthesis, textural properties, in many foods. Likewise, inulin, whether ingested as Jerusalem artichoke tubers or as a bulking agent, is a dietary fibre and confers a number of health advantages, such as, lowers blood cholesterol level (Kaur and Gupta 2002), promotes Bifidobacteria in the large intestine (Hold et al. 2003; Bouhnik et al. 2007).

Chicory (Cichorium intybus), a perennial herb of the Asteraceae family, with blue flowers, lavender, or occasionally white flowers, is native to the Mediterranean region, mid Asia and northern Africa. Historically, chicory was grown by the ancient Egyptians as a medicinal plant. Chicory is a fibre-rich plant with potential prebiotic capacity.

Chicory provides direct functional support to the digestive reactions in the body. Chicory root increases the flow of bile, which supports digestion. Because extra bile helps break down fats, chicory root may help optimize blood composition and is worth consideration by anyone seeking to achieve optimal liver and gallbladder health. Furthermore, organic chicory root contains inulin, a soluble fibre that feeds digestive flora in the intestines.

Figure 2.3.A. The plant (Top) and the tubers (Bottom) of Chicory plant (Cichorium intybus)

Figure 2.3.B.The plant (Top) and the tubers (Bottom) of Chicory plant (Cichorium intybus)

2.3.2. Biological value of Jerusalem artichoke and Chicory

Inulin is a naturally occurring storage polysaccharide present in numerous plants such as Jerusalem artichoke (Judprasong et al. 2011) and chicory root (Mavumengwana 2004). Jerusalem artichoke and Chicory are two plants rich in inulin in their underground parts. Naturally-occurring plant fructans are found as storage carbohydrates in a variety of vegetables including onions, garlic, asparagus and artichokes, in fruits such as bananas, and in cereals (Van Loo et al. 1995). It is not digested or absorbed in the small intestine, but is fermented in the colon by beneficial bacteria. Functioning as a prebiotic, inulin has been associated with enhancing the gastrointestinal tract and the immune system. In addition, it has been shown to increase the absorption of calcium and magnesium, influence the formation of blood glucose, and reduce the levels of cholesterol and serum lipids (Coudray et al. 1997) (Niness 1999).

Chemically, inulin is a linear poly disperse fructan (degree of polymerization, DP, 2–60 or higher) consisting of fructose molecules (F) linked by β (2-1) glycosidic bonds with a terminal glucose molecule (G) connected to the last fructose with a α(1-2) bond (Figure 2.4). These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary fibre effects. Further, these fructans are not hydrolyzed by the digestive enzymes in the small intestine; they reach the colon unabsorbed and are utilized selectively as a substrate for the growth of beneficial

bacteria. Several inulin types occur in nature and they differ in the degree of polymerization and molecular weight, depending on the source, the harvest time, and processing conditions (Chiavaro et al. 2007) (Krivorotava and Jolanta 2014).

The optimum storage conditions of tubers of Jerusalem artichoke can be stored for (6 - 12) months at (0 - 2°C) and (90 - 95%) rate of humidity. Some cultivars are much more susceptible to storage losses than others (Steinbauer 1932).

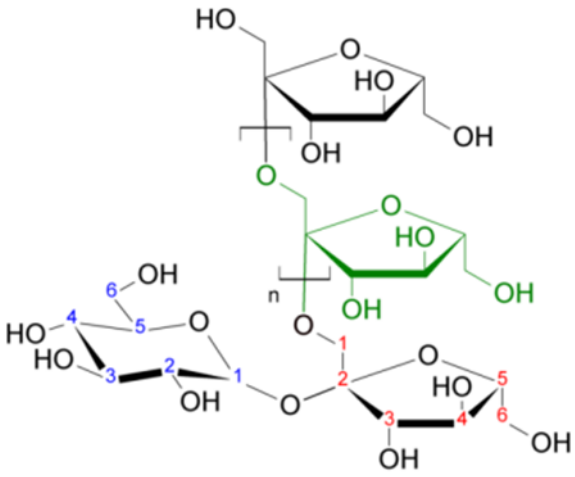

Figure 2.4. Chemical structure of Inulin. (Source: Florianfisch 2006 From Wikimedia, Commons- https://commons.wikimedia.org/wiki/File:Inulin_strukturformel.png)

The tubers of Jerusalem artichoke typically include about 80% water, 17% carbohydrate, and 1 to 2% protein (Kays and Stephen 2008). The principal storage carbohydrate of Jerusalem artichoke is inulin. Jerusalem artichoke tubers have inulin contents of >15% on a fresh weight basis and >75% on a dry weight basis (Kays and Stephen 2008). Gaafar et al. (2010) found that in their study the chemical composition of Jerusalem artichoke, moisture, total carbohydrate, inulin, crude protein, crude fibre and ash were 6.36, 78.03, 72.99, 7.55, 6.51 and 5.72 g / 100 g, respectively. The greater part of carbohydrate that is present in the Jerusalem artichoke present in inulin form.

The findings of determination of inulin in Jerusalem artichoke in the previous study corresponded with other results, but some degree of differences was observed in the levels of inulin. According to Lingyun et al. (2007) showed that the valuable source of inulin can be 14-19% inulin in fresh weight of Jerusalem artichoke tubers, while

Judprasong et al. (2011) showed that the tubers of Jerusalem artichoke contain 16-20% inulin in fresh weight.

Also, Chicory inulin is believed to be a candidate for the prebiotic, which is relatively cheap to manufacture and without any known toxicity (Barbara et al. 2007). The chicory root is one of the most concentrated sources of inulin and oligofructose (Castellini et al. 2007), both of which are classified as prebiotics (Gibson et al. 2004). The root naturally contains approximately 150-200 g/kg inulin and 80-120 g/kg oligofructose (Flickinger et al. 2003). Chicory inulin is described to contain both fructans and oligofructose chains with a degree of polymerization from 11 to 65 and 3 to 10, respectively (Macfarlane et al. 2006).

2.4. Effects of Prebiotics on Poultry

2.4.1. Performance Parameters

There are several reviews discussing the effect of prebiotics on poultry performance. A growing body of scientific research supports the role of prebiotics as effective alternatives to the use of AGP in animal nutrition (Patterson and Burkholder 2003) (Pelicano et al. 2004). The gut microflora affects the digestion, absorption and the metabolism of dietary carbohydrates, protein, lipids and minerals and the synthesis of vitamins (Jin et al. 1997). Most of the volatile fatty acids formed by intestinal bacteria are absorbed and metabolized by the host, contributing to host energy requirements. Maintaining the balance of good gut health is a key aspect of ensuring the best bird performance and health. If an imbalance in gut microbiota occurs, nutrient digestion and absorption may be affected which, in turn, may affect bird health and performance.

Dizaji et al. (2013) showed that the dietary supplementation of Ross 308 broiler with prebiotic (1 kg of ActiveMannanoligosaccharides /ton) had a significant ($P<0.05$) increase on live body weight in prebiotic group compared with the control group at 42 days of experiment. Similarly, Mookiah et al. (2014) showed that use of prebiotic IMO (Wako, Osaka, Japan) in poultry feed significantly ($P<0.05$) improved weight gain of broiler chickens at 22-42 and 1-42 days of age, and feed conversion ratio from 1 to 21,

22-42 and 1-42 days of age compared with control group. Addition of prebiotic to the poultry diets has shown beneficial effects on growth performance of poultry.

2.4.2. Intestinal Microflora

Prebiotics which include non-digestible oligosaccharides may control or manipulate microbial composition and/or activity, there by assisting to maintain a beneficial microflora that suppresses through different regulatory mechanisms the growth of pathogens (Gibson et al. 2004). Prebiotics in the intestinal tract causes the removal of pathogenic bacteria that might attach to the surface of the epithelium cells inside of the intestine (Newman 1994). Oyofo et al. (1989) showed that dietary prebiotic was successful inhibition the intestine colonization of S. typhimurium. Studies on the effects of inulin prebiotic found that foods containing Jerusalem artichoke inulin at the level of 5 g/d significantly increased Bifidobacterium spp. (Ramnani et al. 2010).

Oligofructose or inulin feeding can suppress infections of broilers artificially challenged by salmonella or campylobacter (Van Leeuwen et al. 2005). In an experimental model of necrotizing enterocolitis in quails, the inclusion of oligofructose inhibited the overgrowth of bacteria implicated as pathogens and stimulated the activities of bifidobacteria, which may play a protective role in that case (Catala et al. 1999).

Recently, Akoy (2015) demonstrated that broilers fed with prebiotic had an ability to improve intestinal colonization via decrease E. coli and total aerobic bacteria count in the ileum and caecum digesta than in the control group.

2.4.3. Cholesterol Content

Cholesterol is a critical fatty substance necessary for the proper function of every cell in the body. Cholesterol is a structural component of cell membrane and plasma lipoproteins and is important in the synthesis of steroid hormones and bile acids. Mostly synthesized in the liver, some of it is absorbed through the diet, especially one high in saturated fats (Jaeger and Hedegaard 2004).

According to Yusrizal and Chen (2003), the slowly fermented inulin significantly reduced serum cholesterol levels and deposition of fat tissue in broilers. While, Yalcinkaya et al. (2008) reported that the use of Mannanoligosaccharides from Saccharomyces cerevisiae in broilers diet could not significantly reduce the serum cholesterol levels as compared with the control group. Also, Ashayerizadeh et al. (2011) reported that prebiotic supplementation to the Ross 308 broiler diet at 42 days there was no significant effect in serum cholesterol compared with the control being 3.77 and 4.15 mmol/L, respectively.

2.4.4. Meat Quality

In recent years, the high growth rate, and improvements in meat quality and properties of carcasses have been beneficial to the poultry industry, especially in broiler production and recently Japanese quail. Currently, an important research area is the use of prebiotics as feed additive as an alternative to antibiotics. There are many reports concerning the effect of using prebiotics on feed performance (Abdel-Raheem et al. 2012) (Banday and Risam 2001) (Gunal et al. 2006), but carcass and meat quality of poultry have not been studied.

Colour is an important quality attribute that influences consumer acceptance of many food products, including poultry meat. Consumers will often reject products in which the colour varies from the expected normal appearance. Consequently, colour is often used to determine economic value of food (Qiao et al. 2001).

3. MATERIALS AND METHOD

The study was carried out at the Animal Farm Unit, Department of Animal Science/ Faculty of agriculture/ Bingol University in Turkey, to investigate the effect of two different types of plants as a prebiotic at different concentration to investigate the health benefit on Japanese quail (Coturnix japonica).

3.1. Source of Jerusalem Artichoke And Chicory as Perbiotics

Jerusalem artichoke tubers (*Helianthus tuberosus L*.) were obtained from the local market in Qaladza, Kurdistan-Iraq. The tubers of Jerusalem artichoke were kept in plastic bags and transferred to the laboratory. The tubers were cleaned with tap water to remove dust and other undesirable materials. The cleaned tubers were cut into small pieces and material was dried at 50 °C for 48 h and then ground to a powder and sealed in polyethylene bags. The powdered of Jerusalem artichoke was stored at room temperature, in a dry container to avoid moisture absorption, for further use as recommended by (Lingyun et al. 2007).

Chicory commercially were obtained from online market of Herbal Health Naturally in the United Kingdom as Chicory dried root and kept dried in a plastic bag then cut into pieces and to powder. The powdered kept in dry container and room temperature.

Figure 3.1. The machine that ground the small pieces of plant tubers to a powder

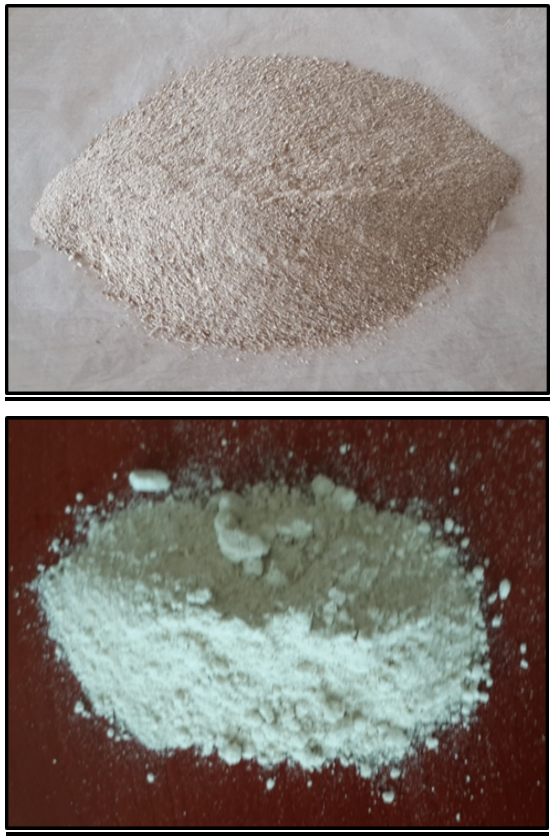

Figure 3.2. The powder of Jerusalem artichoke tubers (Top) and the powder of Chicory tubers (Bottom), which used in this study

3.2. Experimental Design And Treatments

Two hundred and twenty-five one day old Japanese quail were randomly divided into five treatments, fifteen chicks per treatment with three replications.

All treatments were conducted under the same environment including lighting and watering system in a cage house, which it is made by size 90cm × 45cm × 25cm for each replicate. All treatment groups are feeding same diets and rears in cage system for first week after hatch. A commercial diet containing 2900 Kcal/kg and 22% CP was used during the study. These diets were given to the quail's ad libitum. Then, at first day in a second week of age, supplementation dietary was provided as follows: and the project of experiment is shown in Figure 3.3.

Therefore, quails were assigned to the following treatments:
T1= Control group basal Japanese quail diet (no additive)
T2= basal diet + 0.5% dried Jerusalem artichoke
T3= basal diet + 1% dried Jerusalem artichoke
T4= basal diet + 0.5% dried Chicory root
T5= basal diet + 1% dried Chicory root

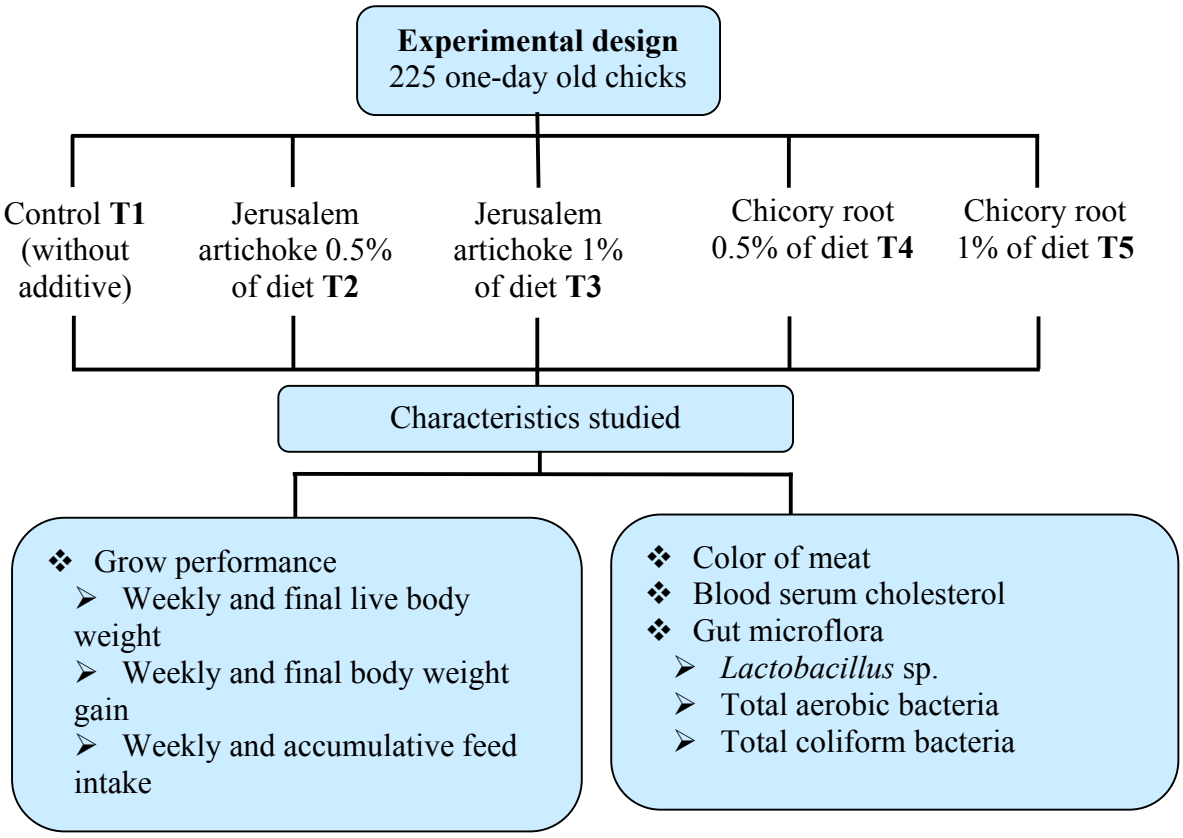

Figure 3.3. Layout of the feed experiment

Figure 3.3. Chicks House located at animal farm unit of Bingol University in Turkey, which used in this study

3.3. Preparing Feed

The commercial diet was used which containing 2900 kcal/kg and 23% CP to quail chicks for 42 days.

3.4. Characteristics Studied

This study was conducted to investigate the effect of dietary Jerusalem artichoke and chicory supplementation on the growth performance, level of blood serum cholesterol and gut microflora of Japanese quail.

3.4.1. Live Body Weight (LBW) And Body Weight Gain (BWG)

At one week old and at the end of each week, birds were weighted by a digital balance. The weight gain was calculated by using this equation:
Weight gain (g) = B.W at the end of the week - B.W at the beginning of the week.

3.4.2. Feed Intake (FI) And Feed Conversion Ratio (FCR)

Feed intake in each replicate was measured and recorded weekly and feed conversion ratio was calculated by the following equation.

$$\text{Feed conversion ratio} = \frac{\text{FI during a feeding period}}{\text{Weight gain during the same period}}$$

3.4.3. Carcass And Body Parts Weight

At the end of study four birds slaughtered, four birds per replicate (two male and two female) and body parts were measured and recorded.

3.4.4. Colour of Meat

A LovibondRT Geries colorimeter (aperture size: 8 mm; light source: illuminant D65) was used to assess the colour [CIE; lightness L*, redness a*, and yellowness b*] of breast

muscles, where L* is the chrome associated to meat lightness, a* is the chrome that ranges between greento red and b* is the chrome that ranges between blue and yellow, according to the methodology proposed by (Pelicano et al. 2005). Standard calibration with black and white tiles was used before measurements. Colour was measured at the surface of male and female breast fillets in an area free from obvious colour defects (bruises, blood spots, or surface discolorations). Meat colors were measured on three birds per treatment and were taken in different position on each sample and the average reading was recorded.

Figure 3.4. A Lovibond colorimeter for colour determination

3.4.5. Blood Serum Cholesterol

In the day 42 of age selected two males and two females from each replicate and slaughtered with sanitized tools. In order to prevent clotting, blood was collected in heparinized test tubes and centrifuged at 2,000 rpm for 10 minutes and then the serum was separated, then stored at -20°C until transferred to central laboratory and assayed to measuring by spectrophotometer and calculated serum cholesterol level by

$$\text{Serum cholesterol level} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X Concentration of Standard}$$

3.4.6. Gut Microflora

At the end of experiment, three birds were selected from each treatment and ileum digesta were aseptically collected to investigate the intestinal microorganisms. The ileum was removed from the carcass under sterile conditions, and immediately transported to the laboratory, Bingol University, Microbiology lab. One hundred milligram of each ileum contents was mixed with 0.9 ml of sterile PBS (pH 7.0) and vortexed for 1 min to homogenize. The homogenate was diluted serially from an initial 10-1 dilution to 10-7. For each dilution 0.1 ml was subsequently plated onto sterile selective medium agar for enumeration of target bacteria groups as following Nutrient agar (Sigma-Aldrich, UK) for total aerobic bacteria, Man, Rogosa and Sharp agar for Lactobacillus spp. and MacConkey agar (Sigma-Aldrich, UK) for total coliform bacteria.

Anaerobic media were incubated in an anaerobic jar with Anarogen (Fisher, England). Man, Rogosa and Sharp agar medium was incubated anaerobically at 37°C for 48 hours. Nutrient and MacConkey agar medium were incubated aerobically at 37°C for 48 hours. The numbers of colonies were then counted to determine the colony forming units (CFU) per gram of fresh ileum digesta using a Colony Counter (Gallenkamp, UK).

3.5. Statistical Analysis

Data obtained were statistically analyzed using one way analysis of variance SAS software 2001 Statistical analyses. Differences among treatment groups were determined by 1-way ANOVA, and means were separated by using Duncan's multiple range test. Treatments were performed in triplicate in each trial. All results were considered significant differences at $P \leq 0.05$ level among the different parameters. Data are presented as mean ± standard error.

The following model was assumed:

$Yij = \mu + Ti + Eij$

Where: μ is the overall mean, Ti is the treatment type, Eij is the random error term.

4. RESULTS

4.1. Live Body Weight And Weight Gain

Live body weight are presented in Table 4.1. There was no significant (P>0.05) difference in initial body weight of Japanese quail among experimental treatments. Also, there were no significant (P>0.05) differences in live body weight of birds among experimental treatments at the first, second, third, fourth, five and six weeks of age, except the male of Japanese quail at the third and fourth weeks of age. The body weight of Japanese quail supplemented with 1% of Jerusalem artichoke as prebiotic was higher than other groups at the end of experiment.

Table 4.2 shows the weekly and average weight gain of Japanese quail during the experiment. There were no significant differences observed among all treatments in weekly weight gain of Japanese quail chicks.

4.2. Feed Intake And Feed Conversion Ratio

Table 4.3 and 4.4 shows the weekly and average feed intake (FI) and feed conversion ratio (FCR) of Japanese quail chicks during the experiment, respectively. The effect of prebiotic supplementations were not significant (P>0.05) on Quail chickens weekly and average feed intake and feed conversion ratio compared with the control group, except the feed intake at second week and feed conversion ratio at second and five week of ages. Also, there were no significant (P>0.05) differences in both types and both levelsof prebiotic in feed intake and feed conversion ratio of Japanese quail. The Quail chicks in Jerusalem artichoke at level 1% groups showed an improvement in final feed conversion ratio compared to all the other the groups, but it is not significant (P>0.05).

Table 4.1. Effect of dietary prebiotics supplementation on weekly and final live body weight (g) of Japanese quail (Means± standard error)

Time	Gender	Treatment					P. value
		T1	T2	T3	T4	T5	
Initial week	--	8.59±0.03	8.50±0.02	8.49±0.02	8.64±0.05	8.68±0.08	0.097
1st week	--	22.10±0.42	22.26±0.34	23.15±0.26	21.88±0.15	21.93±0.45	0.139
2nd week	--	42.52±0.87	42.23±0.84	43.67±1.16	40.72±0.45	42.09±0.49	0.228
3rd week	Male	68.68±0.98 ^{a}	61.05±1.44 ^{b}	69.97±2.74 ^{a}	66.83±1.80 ^{ab}	70.67±2.72 ^{a}	0.049
3rd week	Female	71.11±3.70	71.12±1.63	73.49±5.19	69.40±0.33	67.94±1.98	0.764
4th week	Male	107.77±1.87 ^{ab}	97.05±1.80 ^{c}	107.58±1.72 ^{ab}	104.93±3.84 ^{bc}	114.95±3.15 ^{a}	0.009
4th week	Female	109.30±5.64	108.47±1.88	114.28±8.30	113.65±1.32	107.51±2.20	0.781
5th week	Male	140.84±2.50	133.64±1.35	143.00±2.81	140.52±4.42	145.63±3.16	0.145
5th week	Female	148.46±6.82	147.11±1.49	151.49±9.21	147.57±2.60	144.63±1.78	0.924
6th week	Male	172.45±2.71	167.89±0.96	174.16±1.91	170.10±1.51	174.80±2.13	0.141
6th week	Female	181.97±6.00	181.42±2.59	188.52±9.76	180.25±3.17	182.25±2.38	0.843

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicory and T5: 1% Chico $^{a, b, c}$: means within each row had the different subscript were differ significantly ($P<0.05$)

Table 4.2. Effect of dietary prebiotics supplementation on weekly and final body weight gain (g) of Japanese quail (Means± standard error)

Time	Treatment					P. value
	T1	T2	T3	T4	T5	
1st week	13.50±0.44	13.91±0.37	14.72±0.24	13.24±0.10	13.24±0.50	0.077
2nd week	20.42±1.24	19.96±1.07	20.52±1.37	18.84±0.34	20.16±0.10	0.748
3rd week	27.92±3.03	24.88±1.16	28.22±4.12	27.78±0.40	27.15±0.71	0.857
4th week	38.57±1.72	36.73±0.55	39.85±2.39	39.42±2.42	39.47±0.19	0.719
5th week	36.74±1.35	36.98±1.11	36.90±2.28	36.88±0.33	36.64±0.72	0.999
6th week	32.56±2.75	34.47±0.08	34.72±2.29	31.92±1.22	33.96±1.50	0.768
Final WG	169.73±5.20	166.94±1.04	174.95±6.91	168.10±2.36	170.63±0.62	0.686

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicory and T5: 1% Chicory

Table 4.3. Effect of dietary prebiotics supplementation on weekly and final feed intake (g) of Japanese quail (Means± standard error)

Time	Treatment					P. value
	T1	T2	T3	T4	T5	
1st week	13.89±0.92	12.53±0.17	12.55±0.26	11.56±0.27	11.86±0.50	0.064
2nd week	45.59±2.26 [b]	55.31±1.42 [a]	42.56±2.72 [b]	39.18±3.13 [b]	40.86±3.32 [b]	0.011
3rd week	86.65±6.99	99.74±4.90	87.92±5.69	88.72±10.23	106.40±7.10	0.283
4th week	120.20±5.64	133.61±8.88	105.33±4.28	114.74±10.02	136.61±8.99	0.088
5th week	136.84±7.70	145.64±6.37	119.88±3.81	117.43±6.89	124.70±7.84	0.061
6th week	116.01±7.64	102.46±1.31	111.87±7.26	103.60±2.08	107.76±0.97	0.314
Final WG	519.18±16.84	549.31±15.19	480.11±5.61	475.24±28.17	528.21±25.18	0.103

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicory and T5: 1% Chicory

Table 4.4. Effect of dietary prebiotics on weekly and final feed conversion ratio of Japanese quail (Means± standard error)

Time	Treatment					P. value
	T1	T2	T3	T4	T5	
1st week	1.03±0.07	0.91±0.02	0.86±0.03	0.87±0.01	0.89±0.05	0.147
2nd week	2.22±0.07 [b]	2.78±0.19 [a]	2.09±0.17 [b]	2.08±0.17 [b]	2.02±0.15 [b]	0.039
3rd week	3.17±0.46	4.02±0.23	3.29±0.64	3.19±0.34	3.94±0.36	0.475
4th week	3.14±0.26	3.63±0.19	2.68±0.28	2.92±0.24	3.46±0.21	0.105
5th week	3.73±0.22 [ab]	3.94±0.13 [a]	3.26±0.14 [b]	3.18±0.16 [b]	3.39±0.16 [ab]	0.038
6th week	3.62±0.39	2.97±0.04	3.22±0.06	3.24±0.06	3.19±0.15	0.280
Final FCR	3.06±0.10	3.29±0.07	2.75±0.10	2.83±0.18	3.09±0.15	0.074

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicory and T5: 1% Chicory

4.3. Carcass Quality

Table 4.5 shows the effect of two different types with two levels of prebiotics onJapanese quail chicks during the experiment. There was no significant (P<0.05) difference among all treatments. While, the carcass weight of all dietary additive supplementations mathematically was improved compared with control group, except the Chicory at 0.5% level in male Quail chicks.

Table 4.5. Effect of dietary prebiotics on carcass quality of Japanese quail at the end of experiment (Means± standard error)

Birds	Treatments	Parameters			
		LBW	Carcass weight %	Breast weight %	Leg weight %
Male	T1	174.66±4.25	69.70±1.19	44.20±2.21	26.95±0.90
	T2	168.83±3.92	71.39±1.67	45.82±2.24	27.78±1.31
	T3	172.57±3.74	72.14±1.81	44.96±2.67	28.19±0.92
	T4	170.80±1.00	69.23±2.29	44.94±1.75	27.32±1.16
	T5	171.83±1.20	70.56±0.80	48.38±2.20	27.78±0.90
P. value		0.928	0.986	0.742	0.924
Female	T1	192.66±9.15	68.69±1.09	51.21±2.78	28.94±1.90
	T2	179.33±4.16	70.23±0.64	46.73±1.81	28.63±0.71
	T3	190.14±6.11	71.93±0.27	51.57±2.50	30.26±1.12
	T4	179.20±4.76	73.47±0.96	50.02±3.53	27.99±0.87
	T5	180.16±4.01	69.95±3.37	48.63±3.18	29.98±1.08
P. value		0.348	0.354	0.710	0.684

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicory and T5: 1% Chicory.

4.4. Color of Meat

Table 4.6 shows the colour meat of both sexes of Japanese quail at the end of the experiment.The colour parameters lightness (L* value) of the male was increased significantly in 1% of Jerusalem artichoke and female were increased in 1% of Jerusalem

artichoke and 0.5% of Chicory supplementations compared with other groups. The colour parameters redness (a* value) of the Female were not significant in all additives supplementation compared with control group. While, the colour parameters redness (a* value) of the male were increased in 0.5% of Chicory supplementations compared with other groups.The colour parameters yellowness (b* value) of the male was increased in 1% of Jerusalem artichoke compared with other groups. While, the colour parameters yellowness (b* value) of the femalewas increased in 1% of Jerusalem artichoke and 0.5% of Chicory supplementations compared with other groups. Also, the colour parameter lightness (L* value) of the female wasincreased in 1% of Jerusalem artichoke and 0.5% of Chicory supplementations compared with 0,5% Jerusalem artichoke, 1% of Chicoryand control group.

Table 4.6. Effect of dietary prebiotics on color of meat at 42 days of age of Japanese quail (Means± standard error)

Birds	**Treatments**	**Parameters**		
		L*	**a***	**b***
Male	T1	45.20±1.75 ^{b}	15.48±0.86 ^{ab}	11.51±0.76 ^{ab}
	T2	41.05±2.61 ^{b}	13.27±1.36 ^{b}	9.32±1.36 ^{b}
	T3	54.38±2.26 ^{a}	13.09±0.75 ^{b}	14.09±0.67 ^{a}
	T4	45.94±1.12 ^{b}	16.75±0.28 ^{a}	11.11±1.42 ^{b}
	T5	42.84±2.76 ^{b}	12.64±1.36 ^{b}	8.97±1.04 ^{b}
P. value		0.002	0.049	0.003
Female	T1	46.47±1.11 ^{b}	15.76±0.91	12.65±0.57 ^{a}
	T2	38.09±1.33 ^{c}	12.72±1.28	9.30±0.68 ^{b}
	T3	57.12±1.18 ^{a}	11.92±0.78	14.28±0.36 ^{a}
	T4	56.98±2.32 ^{a}	14.39±1.12	14.39±0.86 ^{a}
	T5	48.19±2.97 ^{b}	12.69±0.99	12.34±1.10 ^{a}
P. value		<0.001	0.073	<0.001

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicory and T5: 1% Chicory.$^{a, b, c}$: means within each row had the different subscript were differ significantly ($P<0.05$).

4.5. Effect of Prebiotic on Gut Microflora

Table 4.7 shows the effects of both types of prebiotic on the microflora in the ileum digesta of Japanese quail at 42 days of age. The results showed that there were no significant (P>0.05) differences between all treatments for total aerobics, Lactobacilli and total coliform at the end of the experiment.

Table 4.6. Effect of dietary prebiotics on bacterial counts (CFU ×105/g) at 42 days of age in ileum digesta of Japanese quail (Means± standard error)

Treatments	**Microbes**		
	Total aerobic	***Lactobacillus* spp.**	**Total Coliform**
T1	73.83±3.49	82.66±8.75	53.33±3.58
T2	72.66±4.27	83.16±5.16	57.33±4.41
T3	79.33±6.10	87.66±5.98	60.33±6.24
T4	72.33±6.84	83.00±6.79	55.50±5.47
T5	80.50±4.32	84.66±2.40	58.16±4.63
P. value	0.690	0.977	0.883

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicoryand T5: 1% Chicory.

4.6. Effect of Prebiotic on Cholesterol Content

Table 4.8 shows the cholesterol of both sexes of Japanese quail at the end of the experiment.There was no significant (P>0.05) differences between all the additives supplementation and compared with control group in both sexes for cholesterol content in total serum.

Table 4.7. Effect of dietary prebiotics on serum cholesterol at 42 days of age of Japanese quail (Means± standard error)

Treatment	Cholesterol content (mg/dl)	
	Male	Female
T1	134.19±4.29	135.91±1.147
T2	130.17±5.73	133.04±3.991
T3	127.87±2.24	134.77±6.302
T4	133.91±4.02	133.90±7.732
T5	122.98±1.60	127.58±4.901
P. value	0.090	0.329

Treatments: T1: control, T2: 0.5% Jerusalem artichoke, T3: 1% Jerusalem artichoke, T4: 0.5% Chicory and T5: 1% Chicory.

5. DISCUSSION

The aim of this study was to investigate the effect of two different types of prebiotic supplementations, Jerusalem artichoke tuber (Helianthus tuberosus) and Chicory roots (Cichorium intybus) on production performance and the gut microflora of Japanese quail. There were no effects of the treatments on BW, but the body weight of Japanese quail supplemented with 1% of Jerusalem artichoke as prebiotic was higher than other groups at the end of experiment. This result agreed with the results of some researchers (Yusrizal and Chen 2003) (Biggs et al. 2007) (Rehman et al. 2008) (and Velasco et al. 2010) (Elrayeh and Yildiz 2012). While, others found that body weight significantly increased by added prebiotic to the diet (Waldroup et al. 1993) (Williams et al. 2008)(and Rebole et al. 2010).

Zhang et al. (2003) showed that prebiotic IMO enhanced growth performance of broiler chickens during the initial 3 weeks, but no further effects were detected during the latter 4 weeks of the experiment. The results studied by Teshfam et al. (2011) who found that adding 1600 g per ton of Fermacto as prebiotic to the diet of Japanese quail significantly ($P<0.05$) higher body weight was observed compared to the control groups at 42 days of age. Similar findings were reported by Mookiah et al. (2014) showed that use of prebiotic IMO (Wako, Osaka, Japan) in poultry feed significantly ($P<0.05$) improved body weight and weight gain of broiler chickens at 22-42 and 1-42 days of age compared with control group.

In literature, the higher performance production observed in poultry fed both types of prebiotic may be due to the fact that additives suppress pathogenic bacteria which lead to improved health status and ultimately improved growth and overall performance. While, in the present study the effects were not observed highly as described in literature.

The results studied by Mookiah et al. (2014) showed that use of prebiotic IMO (Wako, Osaka, Japan) in poultry feed significantly ($P<0.05$) improved feed conversion ratio from1 to 21, 22-42 and 1-42 days of age compared with control group. While, Biggs et al. (2007) reported that 4 or 8 g kg−1 of various prebiotic oligosaccharides (MOS, short-chain FOS, oligofructose, transgalacto-oligosaccharide) had no significant effects on growth performance of young broiler chickens. Similarly, Jung et al. (2008) also reported that the oral administration of prebiotic GOS did not have any significant effect on broiler growth, feed consumption and feed conversion ratio. Also, the results were agreed with El-Abd (2016) who found that there was no significant ($P<0.05$) differences by feed chicory and inulin over the course of the whole experiment compared with the control group in broiler chickens.

The results were agreed with El-Abd (2016) who found that there was no significant ($P<0.05$) differences by feed chicory and inulin over the course of the whole experiment on the carcass characteristics compared with the control group in broiler chickens.

According to previous studies, chicory and Jerusalem artichokes are rich in inulin or oligofructose which they are influencing quail performance. Ammerman et al. (1989) reported that feeding male broilers a 0.375% level of oligofructose produced heavier birds at 47 days and improved hot carcass weight percent and breast weight percent, while fat pad percent was decreased. The study performed by Yusrizal and Chen (2003) which showed that the addition of inulin and FOS at 10 g/kg of diet improved body and carcass weights in female broiler chickens.

Colour is an important quality attribute that influences consumer acceptance of many food products, including poultry meat. Consumers will often reject products in which the colour varies from the expected normal appearance. Consequently, colour is often used to determine economic value of food (Qiao et al. 2001). The variations in colour of meat fillets were significant correlated with muscle pH and extremes in colour variations. Meat may appear dark due to high muscle pH (Karaoglu et al. 2004).

In this experiment the additives supplementations were used which increased lactic acid and this may decrease the pH value in the intestine which may be increasing the lightness

(L* value) and yellowness (b* value). In this study, the pH was not measured, so no conclusion can be made. Because, Lightness (L*) and yellowness (b*) were found to correlate negatively to pH, whereas redness (a*) had a positive correlation (Salakova, et al. 2009).

The population of the intestinal bacterial community can be changed by a variety of factors, diet being one of them as it acts as a substrate for the indigenous intestinal microflora (Rehman et al. 2007). Prebiotic can stimulate the growth of the intestinal bacteria as well as alter the ratio of various SCFA (Rehman et al. 2008). Gut Flora consists of a complex community of microorganisms that live in the digestive tracts of animals. It is documented that dietary fructans influence the intestinal gut microflora of Quail by increasing the population of Bifidobacterium spp., Lactobacillus spp. and Eubacterium spp. while decreasing the concentration of Clostridium spp. and Escherichia coli in the large intestine and caeca. Akoy (2015) reported additive of probiotic, prebiotic and synbiotic supplementation increase the numbers of total anaerobic, Lactobacillus spp. and Bifidobacterium spp. compared with control group at 17 and 35 days of age at the same time the numbers of total aerobic bacteria and total coliform in all additives supplementation are decreases in the ileum digesta.

Park and Park (2012) demonstrated that the growth of Bifidobacterium spp. and Lactobacillus spp. in caecum was stimulated by adding inulin (Prebiotic) to the diet compared with the control group, while the growth of E. coli and Salmonella was clearly inhibited ($P<0.05$). Similarly, Rebole et al. (2010) showed that Bifidobacteria in broilers caecum digesta in laying hens significantly increased after adding inulin to their diets.

The normal gut microflora in farm animals is important because of its effect on the production of livestock and the quality and safety of livestock products. In poultry, the caecal microflora can protect chickens against bacterial infection; a healthy microflora present in the small intestine contributes significantly to small intestinal function, including digestion and nutrient absorption (Gil De Los Santos et al. 2005) (Mountzouris et al. 2007).

Prebiotic related carbohydrates are not dissolved in the small intestine of birds and reached the lower part of digestive system where it reduces the numbers of harmful microorganisms E. coli and Salmonella and selectively promotes the growth of beneficial microorganisms lactobacilli and bifidobacteria (Yusrizal and Chen 2003) (Park and Park 2011). One other reasons to reduce the number of pathogens, they could attached with the prebiotics instead of attaching to intestinal epithelial cells and, therefore, move through the intestine without colonization (Newman 1994).

Kaur and Gupta (2002) reported that the dietary fibre and confers a number of health advantages, which it causes to decrease blood cholesterol level. Yusrizal and Chen (2003) reported that adding inulin or oligofructose as prebiotic at the level of 1% reduced ($p<0.05$) serum cholesterol of broilers. Also, this result was disagreed with El-Abd (2016) who found that cholesterol content was decreased by 3% and 6% of Jerusalem artichoke as prebiotic supplementation compared with control group. This may due to inulin play a role in modulation of liver enzymes. Similarly, they attributed this reduction to the cholesterol assimilation to the Lactobacilli or to the co-precipitation of cholesterol with deconjugated bile salts (Gilliland et al. 1985).

The effects of prebiotic on serum cholesterol concentrations are inconsistent among previous studies. Some studies have shown that prebiotic exhibited lipid-lowering properties which might be related to the changes in the intestinal bacterial flora composition, which ferments prebiotics to produce short-chain fatty acids in the gut and then causes a decrease in the systemic levels of blood lipids and cholesterol. Another explanation to these inconsistent results might be because of the level of dose used and the period of time administered as well as type of prebiotic (Angel et al. 2005) (Patterson and Burkholder 2003).

6. CONCLUSIONS AND FUTURE WORK

Conclusion

Prebiotic can stimulate the growth of the intestinal bacteria as well as may effect on the growth performance. The aim of this study was to investigate the influence of prebiotic from Chicory and Jerusalem artichoke tubers (Helianthus tuberosus) on the growth performance, ileum microflora and cholesterol content of Japanese quail chicks. No significant differences were observed between both types of prebiotic treatments and compared with control group for all parameters, except the breast meat colour at the end of the experiment.

Future work

The main areas to be highlighted are:

1. The research is need to increase knowledge regarding the effect of various levels of prebiotics to evaluate their effects on the growth performance, modulate the composition of gut microflora and histology of small intestine.
2. There is some knowledge accumulated on the application of prebiotic in the small intestine of poultry industry but this is still limited and the research should continue. For example, little is known about the immunological response of the chicken to prebiotics.
3. The research is needed to increase the level of prebiotic instead of 1% of Jerusalem artichoke to show the effect of prebiotics on performance and gut microflora.

REFERENCES

Abdel-Raheem SM, Sherief MS, Khaled MA (2012) The Effects of Prebiotic, Probiotic and Synbiotic Supplementation on Intestinal Microbial Ecology and Histomorphology of Broiler Chickens. International Journal for Agro Veterinary and Medical Sciences 6(4): 277-289

Akoy RAM (2015) the effects of probiotics, prebiotics and synbiotics on gut flora, immune function and blood characteristics of broilers. PhD Thesis, School of Biological Sciences, Faculty of Science and Engineering, Plymouth University.19

Ammerman E, Quarles C, Twining PV (1989) Evaluation of fructooligosaccharides on performance and carcass yield of male broilers Poultry. Science 68: 167-19

Angel R, Dalloul RA, Doerr J (2005) Performance of broiler chickens fed diets supplemented with a Direct-Fed Microbial. Poultry Science 84: 1222-1231

Ashayerizadeh A, Dabiri N, Mirzadeh KH, Ghorbani MR (2011) Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. Journal of Cell and Animal Biology 5(8): 152-156

Awad WA, Ghareeb K, Bohm J (2011) Evaluation of the chicory inulin efficacy on ameliorating the intestinal morphology and modulating the intestinal electrophysiological properties in broiler chickens. Journal of Animal Physiology and Animal Nutrition95: 65-72

Banday MT, Risam KS (2001) Growth performance and carcass characteristics of broiler chicken fed with probiotics. Indian Journal of Poultry Science 36: 252-255

Biggs P, Parsons CM, Fahey GC (2007) the Effects of Several Oligosaccharides on Growth Performance, Nutrient Digestibilities, and Cecal Microbial Populations in Young Chicks. Poultry Science 86:2327-2336

Brighenti F, Pellegrini N, Casiraghi M, Testolin G (1995) In vitro studies to predict physiological effects of dietary fibre. European Journal of Clinical Nutrition 49 pp S81 (8)

Bouhnik Y, Achour L, Paineau D, Riottot M, Attar A, Bornet F (2007) Four-week short chain fructo-oligosaccharides ingestion leads to increasing fecal bifidobacteria and cholesterol excretion in healthy elderly volunteers. Nutrition Journal6: 42

Castellini C, Cardinali R, Rebollar P, Dal Bosco A, Jimeno V, Cossu M (2007) Feeding fresh chicory (Chicoria intybus) to young rabbits: Performance, development of gastro-intestinal tract and immune functions of appendix and Peyer's patch. Animal Feed Science and Technology 134: 56-65

Chiavaro E, Vittadini E, Musci M, Bianchi F, Curti E (2008) Shelf-life stability of artisanally and industrially produced durum wheat sourdough bread (Altamura bread). LWT-Food Science and Technology 41: 58-70

Connell I, Agace W, Klemm P, Schembri M, Mărild S, Svanborg C (1996) Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. Proceedings of the National Academy of Sciences 93: 9827-9832

Coudray C, Bellanger J, Castiglia-Delavaud C, Remesy C, Vermorel M, Rayssignuier Y (1997) Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium,magnesium,iron and zinc in healthy young men. European Journal of Clinical Nutrition51: 375-380

Crittenden R, Karppinen S, Ojanen S, Tenkanen M, Fagerström R, Mättö J, Saarela M, Mattila-Sandholm T, Poutanen K (2002) In vitrofermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. Journal of Science Food Agriculture 82: 781-789

Dizaji BR, Afshin Z, Amirhossein G, Saman F, Orhan R (2013) Influences of different growth promoters on intestinal morphology of broiler chickens. European Journal of Experimental Biology 3(2):32-37

El-Abd NM (2016) Efficiency of Jerusalem artichoke Tubers Powder as Feed Additives on Japanese Quail Performance. Egyptian Poultry Science Journal 36: 1-9

Elrayeh AS, Yildiz G (2012) Eff ects of inulin and β-glucan supplementation in broiler diets on growth performance, serum cholesterol, intestinal length, and immune system. Turkish Journal of Veterinary and Animal Sciences 36(4): 388-394

Flemming JS, Freitas JRS, Fontoura P, Montanhini NR, Arruda JS (2004) Use of mannanoligosaccharides in broiler. feeding, Brazilian Journal of Poultry Science6(3):159-161

Flickinger EA, Loo JV, Fahey GC (2003) Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals a review Critical Reviews in Food Science and Nutrition 43: 19–60

Fooks LJ, Fuller R, Gibson GR (1999) Prebiotics, probiotics and human gut microbiology. International Dairy Journal 9: 53-61

Gaafar A, Boudy E, El-Gazar H (2010) Extraction conditions of Inulin from Jerusalem artichoke tubers and its effects on Blood glucose and Lipid Profile in diabetic rats. Journal of American Science 6: 36-43

Gibson G, Roberfroid M (1995) Dietary modulation of the human colonic microbiota introducing the concept of prebiotics. The Journal of Nutrition 125: 1401-1412

Gibson GR, Probert HM, Van Loo J, Rastall RA, Roberfroid MB (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews 17: 259-275

Giggs JP, Jacob JP (2005) Alternatives to antibiotics for organic poultry production. Journal of Applied Poultry Research 14: 750-756

Gil De Los Santos JR, Storch OB, Gil-Turnes C (2005) Bacillus cereus var. toyoii and Saccharomyces boulardii increased feed efficiency in broilers infected with Salmonella Enteritidis. British Poultry Science 46: 494-497

Gilliland SE, Nelson CR, Maxwell C (1985) Assimilation of cholesterol by Lactobacillusacidophilus. Applied and Environmental Microbiology 49: 377–381

Gunal M, Yayli G, Kaya O, Karahan N, Sulak O (2006) the effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. International Journal of Poultry Science 5: 149-155

Hillman K (2001) Bacteriological Aspects of the Use of Antibiotics and Their Alternatives in the Feed ofNon-ruminant Animals In, Recent Advances in Animal Nutrition, Garnsworthy, PC. and J. Wiseman (Eds) Nottingham University Press, Nottingham pp: 107-134

Hold GL, Schwiertz A, Aminov RI, Blaut M, Flint HJ (2003) Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces Applied and Environmental Microbiology. 69: 4320-4324

Jin LZ, Ho YW, Abdullah N, Jalaludin S (1997) Probiotics in poultry modes of action. World's Poultry Science journal 53: 351-368

Judprasong K, Siriporn T, Prapasri P, Pongtorn S (2011) Investigation of Thai plants for potential sources of inulin-type fructans. Journal of Food Composition and Analysis 24: 642-649

Jung SJ, Houde R, Baurhoo B, Zhao X, Lee BH (2008) Effects of galactooligosaccharides and a Bifidobacteria lactis-based probiotic strain on the growth performance and fecal microflora of broiler chickens. Poultry Science87:1694-1699

Kaur N, Gupta AK (2002) Applications of inulin and oligofructose in health and nutrition. Journal of Biosciences 27: 703-714

Kays SJ, Stephen FN (2008) Biology and Chemistry of Jerusalem Artichoke Helianthus tuberosus L. CRC Press, Taylor, Francis Group. Boca Raton, FL 33487-2742

Kleessen B, Elsayed NAAE, Loehren U, Schroedl W, Krueger M (2003) Jerusalem artichokes stimulate growth ofbroilers chickens and protect them against endotoxins andpotentialcecal pathogens. Journal Food Protection 66: 2171-2175

Krivorotava T, Jolanta S (2014) Seasonal changes of carbohydrates composition in the tubers of Jerusalem artichoke. Acta Physiologiae Plantarum 36: 79-83

Lingyun W, Wang J, Zheng X, Teng D, Yang Y, Cai C, Feng T, Zhang F (2007) Studies on the extracting technical conditions of inulinfrom Jerusalem artichoke tubers. Journal of Food Engineering79: 1087-1093

Liu H (2008) Influence of chicory feeding on performance and gut development in broilers, M.Sc. Thesis, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences

Macfarlane S, Macfarlane GT (2006) Composition and metabolic activities of bacterial biofilms colonizing food residues in the human gut. Applied of Environmental Microbiology 72: 6204-6211

Mavumengwana VB (2004) Isolation, purification and characterization of inulin and fructooligosaccharides from chicorium intybus and inulinase from aspergillus Niger. Master of Science, Rhodes University

Mookiah S, Chin CS, Kalavathy R, Norhani A, Yin WH (2014) Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. Journal of Science and Food Agriculture 94(2): 341-348

Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K (2007) Evaluation of the Efficacy of a Probiotic Containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus Strains in Promoting Broiler Performance and Modulating Cecal Microflora Composition and Metabolic Activities. Poultry Science 86: 309-317

Newman K (1994) Manna-oligosaccharides: Natural polymers with significant impact on the gastrointestinal microflora and the immune system. Pages 167-174 in: Biotechnology in the Feed Industry.Proceedings of Alltech's 10th Annual Symposium, TP. Lyons and KA. Jacques, eds. Nottingham University Press, Nottingham, United Kingdom

Niness KR (1999) Inulin and oligofructose, What are they?. Journal of Nutrition 129: 1402S–1406S

Oyofo BA, DeLoach JR, Corrier DE, Norman JO, Ziprin RL, Molenhauer HH (1989) Prevention of Salmonella typhimurium colonization of broilers with D-mannose. Poultry Science 68:1357-1360

Patterson JA, Burkholder KM (2003) Application of prebiotics and probiotics in poultry production. Poultry Science 82: 627-631

Park SO, Park BS (2011) Effect of dietary microencapsulated-inulin on carcass characteristics and growth performance in broiler chickens. Journal of Animal and Veterinary Advances 10: 1342-1349

Park SO, Park BS (2012) Effect of feeding inulin oligosaccharides on cecum bacteria, egg quality and egg production in laying hens. African Journal of Biotechnology 11(39): 9516-9521

Pelicano ERL, Souza PA, Souza HBA, Figueiredo DF, Boiago MM, Carvalho SR, Bordon VF (2005) Intestinal mucosa development in broiler chickens fed natural growth promoters. Brazilian Journal of Poultry Science 7(4): 221- 229

Qiao M, Fletcher DL, Smith DP, Northcutt JK (2001) the effect of broiler breast meat color on pH, moisture, water holding capacity, and emulsification capacity. Poultry Science 80: 676-680

Ramnani P, Gaudier E, Bingham M, Bruggen P, Touhy KM, Gibson GR (2010) prebiotic effect of fruit and vegetables shots containing Jesusalem artichock inulin a human intervention study. British Journal of Nutrition 104(02): 233- 240

Rebole A, Ortiz LT, Rodriguez ML, Alzueta C, Trevino J, Velasco S (2010) Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat- and barley-based diet. Poultry Science 89: 276-286

Rehman H, Hellweg P, Taras D, Zentek J (2008) Effects of dietary inulin on the intestinal short-chain fatty acids and microbial ecology in broiler chickens as revealed by denaturing gradient gel electrophoresis. Poultry Science 87:783-789

Roberfroid MB (2007) Inulin type fructans: Functional food ingredients. Journal of Nutrition137: 2493S-2502S

Salakova A, Eva S, Veronika V, Hana B, Iva S (2009) Quality Indicators of Chicken Broiler Raw and Cooked Meat Depending on Their Sex. Acta Veterinaria Brno78: 497-504

Salminen S, Bonley C, Bourron-Ruanlt M, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau MC, Roberfroid M, Rowland I (1998) Functional food science and gastrointestinal physiology and function. British Journal Nutrition 80: 147S-171S

Slimestad R, Slejaasen R, Meijer K, Skar SL (2010) Norwegian growth Jerusalem artichoke (*Helianthus tuberosus L.*) morphology and content of sugars and fructo-oligosaccharides in stems and tubers. Journal Science Food Agriculture 90: 956-964

Sofia K, Gibson GR (2007) Prebiotic Capacity of Inulin-Type Fructans. The Journal of Nutrition 137: 2503S-2506S.

Steinbauer CE (1932) Effects of temperature and humidity upon length of rest period of tubers of Jerusalem artichoke (*Helianthus tuberosus*). Proceedings of the American Society for Horticultural Science 29:403-408

Stolzenburg K (2005) Jerusalem artichokes-raw material for inulin and fructose production. Zuckerindustrie 130: 193-200

Van Loo J, Coussement P, Deleenheer L, Hoebregs H, Smits G (1995) on the presence of inulin and oligofructose as natural ingredients in the western diet. Crit. Rev. Food Science and Nutrition 35: 525-552

Velasco S, Ortiz LT, Alzueta C, Rebole A, Trevino J, Rodriguez ML (2010) Effect of inulin supplementation and dietary fat source on performance, blood serum metabolites, liver lipids, abdominal fat deposition, and tissue fatty acid composition in broiler chickens. Poultry Science 89: 1651-1662

Verdonk JMAJ, Van Leeuwen P (2004) the application of inulin-type fructans in diets for veal calves and broilers. In Inulin and oligofructoseFeelgood Factors for health and well-being, 4th Orafti Research Conference, Paris, February 12-13: 50-51

Waldroup AL, Skinner JT, Hierholzer RE, Waldroup PW (1993) an evaluation of fructooligosaccharide in diets for broiler chickens and effects on salmonellae contamination of carcasses. Poultry Science 72: 643-650

Williams J, Mallet S, Lacontem M, Lessire M, Gabriel J (2008) The effects of fructooligosaccharides or whole wheat on the performance and digestive tract of broiler chickens. British Poultry Science 49:329-339

Yalcinkaya H, Gungori T, Bafialani M, Erdem E (2008) Mannan oligosaccharides (MOS) from Saccharomyces cerevisiae in broilers, effects on performance and blood biochemistry. Turkish Journal of Veterinary and Animal Sciences32(1): 43-48

Yusrizal, Chen TC (2003) Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length. International Journal of Poultry Science 2: 214-219

Zhang, WF, Li DF, Lu WQ, Yi GF (2003) Effects of isomalto-oligosaccharides on broiler performance and intestinal microflora. Poultry Science 82:657–663

Ziemer CJ, Gibson GR (1998) an overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. International Dairy Journal8: 473-479

APPENDIX

Table A.1. Analysis of active compound in the tubers of Jerusalem artichoke (Helianthus tuberosus) by GC-MS

Peak no.	RT	Area %	Identified Compound
1	15.535	2.22	Dichloroacetic acid
2	16.936	7.35	Bicyclooct-2-ene
3	19.431	35.05	CYCLOHEXENE
4	19.992	3.65	1,9-Nonanediol
5	21.262	0.94	Oxalic acid
6	21.520	1.80	3-Cyclopentene-1-acetaldehyde
7	21.663	2.16	Octane
8	22.332	1.09	Undecane
9	22.550	9.25	TETRACOSANE
10	22.790	0.89	Oxirane
11	23.912	1.18	1-Pentanol
12	24.015	3.86	Octane
13	24.278	0.80	DECANE
14	28.724	1.78	Dodecane
15	32.077	24.57	CYCLOHEXENE
16	37.644	1.62	PANASINSENE<BETA->
17	43.343	1.80	Furo pyridine

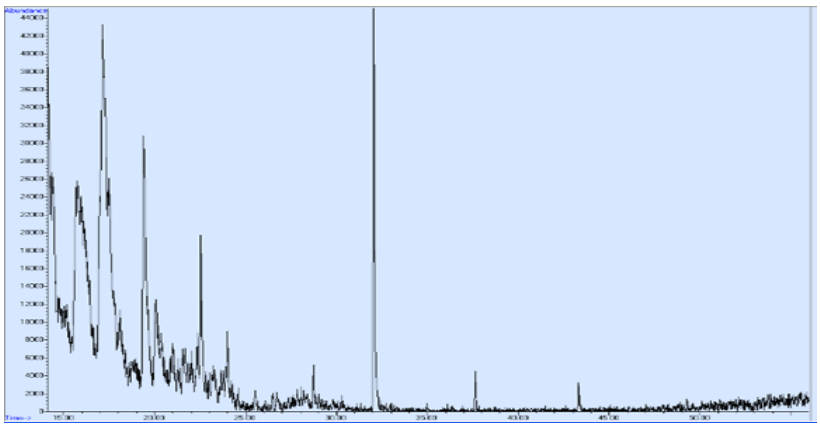

Figure A.1. Analysis of active compound in the tubers of Jerusalem artichoke by GC-MS

Table A.2. Analysis of active compound in the tubers of Chicory (Cichorium intybus) by GC-MS

Peak no.	RT	Area %	Identified Compound
1	15.672	10.35	FURAN
2	18.058	3.69	BETA-PHELLANDRENE
3	19.431	69.55	3,7,7-TRIMETHYLBICYCLOHEPT-3-ENE
4	19.998	5.28	2-HEPTENE
5	22.349	2.28	Oxalic acid
6	22.544	4.10	Tetradecane
7	23.654	2.23	4-METHOXY-2-BUTYN-1-OL
8	23.912	2.53	Acetic acid

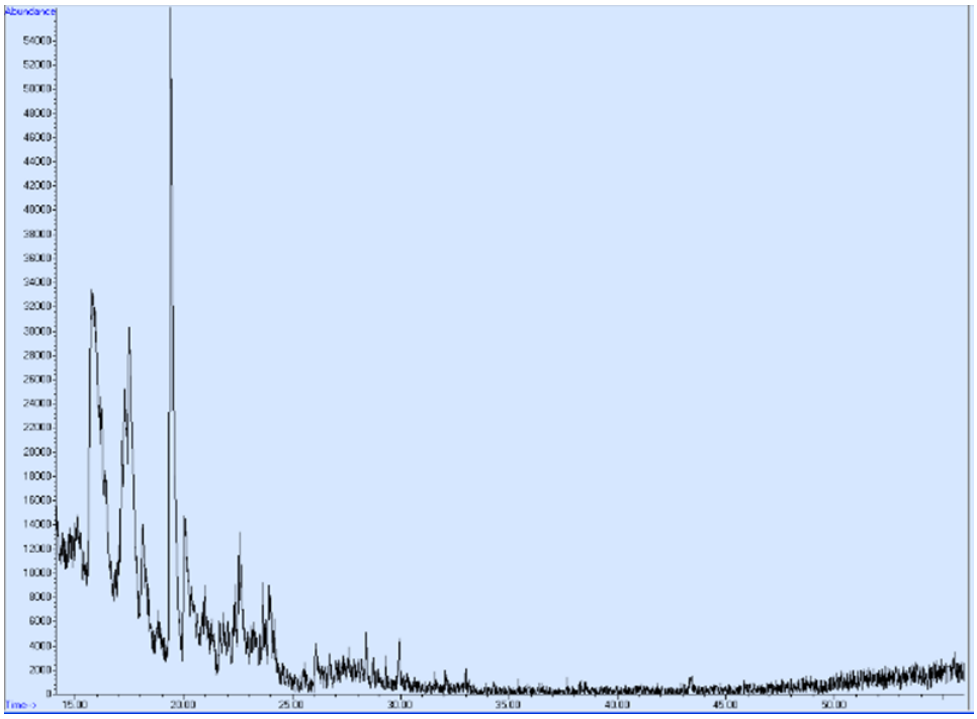

Figure A.2. Analysis of active compound in the tubers of Jerusalem artichoke by GC-MS

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