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EFFECT OF ANIMAL FEED SUPPLEMENTATION WITH DIFFERENT CONCENTRATIONS OF FENUGREEK SEEDS (*Trigonella foenumgraecum*) ON ANIMAL PRODUCTION AND MICROBIAL CHICKEN MEAT.

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ABSTRACT

This study was conducted in the poultry field of Al-Mustansiriya University/ Baghdad, to show the effect of adding different levels 0, 1, 5 and 10% of the fenugreek seeds in the rations containing many contaminated microbes on the productive performance of broilers. 150-day-old rose meat was used with a 41 average weight (gm), were randomly allocated to 4 treatments with 3 replicates, and for each treatment 15 chicks per repetition: 0, 1, 5, and 10% (T0-T3), respectively. The results of this study showed that fenugreek seeds contain good amounts of protein, fat, ash and carbohydrates, which are 24.92, 8.82, 3.08 and 54.28 respectively. Fenugreek seeds also have high levels of tannins, coumarins and flavones, followed by saponins, alkaloids, resins and starch. While the fenugreek seeds were distinguished by their lack of glycosides. In addition, the fenugreek was characterized by containing significant quantities of fatty acids such as oleic, linoleic and linolenic acids by 32.54, 55.04 and 4.55% respectively. The results of this study showed significant superiority ($P < 0.05$) for treatment T2 from adding seeds Fenugreek over the rest of the transactions in the average body weight, weight gain and food conversion factor. As for the feed conversion factor, the T2 treatment also recorded (adding 5% of fenugreek seeds) a significant improvement ($P < 0.05$) compared to the control treatment. The results showed a significant superiority of ($P < 0.05$) in the measure of the productive evidence for the T2 treatment compared to the control treatment. Followed by the addition of 1% of fenugreek seeds had a moderate significant increase, while an increase of the addition rate of 10% decreased the weighted production percentage of chicks with a significant decrease compared to the control treatment.

Keywords: Fenugreek seeds, chemical composition, productive traits, microbial contamination.

DOI: [http://dx.doi.org/10.28936/jmraipc12.2.2020.\(7\)](http://dx.doi.org/10.28936/jmraipc12.2.2020.(7))تأثير تدعيم العلف الحيواني بتركيز مختلفة من بذور نبات الحلبة في الانتاج الحيواني والميكروبي لدجاج اللحم
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الخلاصة

أجريت هذه الدراسة في حقل الدواجن التابع للجامعة المستنصرية في مدينة بغداد وذلك لبيان تأثير إضافة مستويات مختلفة صفر و 1 و 5 و 10% من مطحون بذور الحلبة في العلائق المحتوية على الكثير من المايكروبات الملوثة في الأداء الإنتاجي لفروج اللحم، إذ تم استخدام 150 فرخ لحم جنس نوع روز بعمر يوم واحد وبمعدل وزن 41 غم، وزعت عشوائياً على 4 معاملات وبواقع 3 مكررات ولكل معاملة 15 فرخ لكل مكرر صفر و 1 و 5 و 10% (T0-T3) على التوالي، وبينت نتائج هذه الدراسة احتواء بذور الحلبة على كميات لا بأس بها من البروتين والدهن والرماد والكاربوهيدرات وهي 24.92 و 8.82 و 3.08 و 54.28 على التوالي، وكذلك تمتاز بذور الحلبة باحتوائها على كميات عالية من التانينات والكومارينات والفلافونات ويليها الصابونينات والقلويدات والراتنجات والنشا، بينما تميزت بذور الحلبة بخلوها من الكلايكوسيدات، فضلاً عن ذلك تميزت الحلبة باحتوائها على كميات لا بأس بها من الأحماض الدهنية كاحماض الأوليك واللينوليك واللينولينك بنسبة 32.54 و 55.04 و 4.55% على التوالي، وأظهرت نتائج هذه الدراسة التفوق المعنوي ($P < 0.05$) للمعاملة T2 من إضافة بذور الحلبة على بقية المعاملات في معدل وزن الجسم والزيادة الوزنية ومعامل التحويل الغذائي، أما فيما يتعلق بمعامل التحويل الغذائي فقد سجلت المعاملة T2 أيضاً (إضافة 5% من بذور الحلبة) تحسناً معنوياً ($P < 0.05$) مقارنة مع معاملة السيطرة، وقد أظهرت النتائج تفوق معنوي ($P < 0.05$) في مقياس الدليل الإنتاجي للمعاملة T2 مقارنة بمعاملة السيطرة والمعاملتان T1 و T3 (إضافة 1 و 10% من بذور الحلبة). الكلمات المفتاحية: بذور الحلبة، التركيب الكيميائي، الصفات الانتاجية، التلوث الميكروبي.

INTRODUCTION

Medicinal herbs have a special place in agricultural production for their apparent effect on the overall, physiological and therapeutic production of humans and animals, especially poultry (**Bash et al., 2003**). Also, medicinal herbs are the main source of many medicinal drugs used for various therapeutic purposes, such as treating many chronic diseases, as they are a source of effective substances that go into preparing many medicinal compounds in the form of extracts or other forms (**Abdouli et al., 2014**). Among these medicinal plants are fenugreek *Trigonella foenum-graecum*, which is one of the important plants and commonly used in medicine since ancient times and is used today widely in all parts of the world, and the importance of fenugreek seeds is due to its contain of active substances such as alkaloids, cyclosides, flavonoids, saponins and volatile oils...etc. from other ingredients as well as the basic constituents like protein, fats, vitamins, and rare minerals (**Hein et al., 2018**), fatty acids, amino acids, vitamins and mineral elements (**Barnes et al., 2002; Langmead et al., 2002; Pandian et al., 2002**). Fenugreek hay was used as animal feed by the Greeks who called it fenugreek hay. The Indian Research Association (**Ozan et al., 2011**) stated that most veterinarians recommend using fenugreek in animal feed to increase disease resistance and improve animal weight production. Experiments have proven that fenugreek is an appetite suppressant, which is reflected in the increased appetite for eating food and thus achieving an



increase in the average body weight of the body (Jae-Hong *et al.*, 2018; Park & Kim 2016). Because of the important effects of fenugreek seeds on the productive characteristics of broilers, this study came to know the effect of adding different concentrations of fenugreek seeds in strengthening poultry feed on the rate of weight gain, feed consumption, conversion efficiency, and evaluation of pathogenic bacteria in poultry feed and its resistance to antibiotics, due to eat fenugreek seeds.

MATERIALS AND METHODS

5 kg of fenugreek seeds were brought which available in the local markets of Baghdad / Karkhside. The plant variety in the Department of Biology College of science, Al-Mustansiriya University, which confirmed its gender and type, is *Trigonella foenum-graecum* and belongs to the family of *Fabaceae* and the English name is Fenugreek and its Arab name is Al-hilba. Fenugreek seeds were purified from impurities and gravel, and after that fenugreek seeds were ground by the electric mill available in the laboratory to a very fine powder for the animal to benefit from its consumption. Then the powder was kept in a sealed glass container and kept in a cool place and away from sunlight until use.

Determination some of the main ingredients of fenugreek seeds

The main components of fenugreek seeds were estimated moisture, protein, fat, ash and carbohydrates according to AOAC (1980).

Preparing the aqueous extract of fenugreek seeds for phytochemical detection

10 g of dry fenugreek seed was weighed and placed in a 500 mL glass beaker and 200 mL of distilled water added and mixed with a magnetic mixer for 15 min. and then left for stirring for 30 min. to precipitate the insoluble vegetable parts, then the solution was filtered and the filtrate was taken and separated by centrifuge At a speed of 3000 cycles / for a period of 10 min., to settle the remaining suspended minutes again to obtain a pure solution, and then concentrate the solution in the inner laboratory at a temperature of 40°C until the solution was completely dried and the dryer was kept in a dry and sealed container until the phytochemical disclosures were made.

Detection of phytochemical compounds in fenugreek seeds

Specific estimations were made for claycides, saponins, tannins, alkaloids, resins, coumarins, flavones and starch for fenugreek seeds according to Hashemi *et al.*, (2008).

Separation of fat and fatty acids

Volatile oils were extracted using the continuous extraction method by using the Soxhlet and using ether as a solvent, as 5 gm of ground fenugreek seeds were laid pre-prepared with the addition of 250 mL of ether. Dried and opaque bottles until fatty acid is analyzed by the GC-Mass (Wang *et al.*, 2000) device at the General Establishment for Vegetable Oils laboratories in Baghdad.

Determination of bacterial load

TVC examination was performed according to the recommendation of NRC (1994).

Study the inhibitory activity of fenugreek seed extract in different genera of bacteria

In this part of the study, the effect of the active substances in fenugreek seed meal on the growth of five genera was studied from the following bacteria:

1. *Esherichia spp.*
2. *Pasteurella spp.*
3. *Bacillus spp.*
4. *Staphylococcus aureus*
5. *Salmonella spp.*



Diffusion method was used around digging the Agar plates, and the diagnosed bacterial isolates were obtained from Yarmouk Hospital in Baghdad. The sensitivity was tested for the Bauer test for antibiotics, as it followed the method of bacterial allergy to 5 types of antibiotics and prepared by oxide, as it transported 4-5 pure colonies of bacteria to the center of the broth, and incubated bacterial cultures at 37°C for 14-16 hours and then diluted the suspension. Bacterial with physiological saline, then took 0.1 mL of bacterial trap and spread on the surface of a dish containing a Muller Hinton medium using a sterile cotton swab, the dishes were left at room temperature for 30 min., then the antibiotic tablets were stabilized by sterile forceps, incubated dishes at 37°C for a period 24 hours, then the area of growth inhibition was measured in (mm).

Feed reinforcement

This study was conducted at the Animal and Fisheries Center, Agricultural Research Department, Al-Mustansiriyah University, in the city of Baghdad for a period of 45 days. In this experiment, 150 Turkish origin meat chicks were used at the age of one day, with an average weight of 52 gm, distributed over 3 treatments, with 15 chicks per treatment, as follows:

1. Basic diet free from any additives as control treatment = T 0.
2. Basic feed added 1% ground fenugreek seeds = T 1.
3. Basic diet added to 5% of the fenugreek seed meal = T 2.
4. Basic diet added to 10% ground fenugreek seeds = T 3.

Body weight, total weight gain, feed consumption and conversion factor were calculated.

The experiment

Upon the arrival of the chicks in the prefabricated animal field hall, the chicks were vaccinated directly with the Even vaccine and the record of the preventive program followed by the vet was followed. Then the chicks were fed the length of the experiment free feeding, i.e. from the age of one day to the age of 21 days on a starter diet containing 20.19% of protein and 3589 kcal per kg of cal per 1 kg of feed until the end of the experiment as shown in (Table 1), taking into account the availability health care and ventilation needed during the trial days. The weights of the chicks were taken individually, for each treatment and for all replicas designed in this study, at the age of one day, as well as at the end of each week and up to six weeks. Then the weight gain, the feed consumption rate, the feed conversion factor and the total mortality ratio were calculated according to what he mentioned **Al-Aqil (2016)** and then the food conversion factor was calculated as in the following equation and according to **Park & Kim (2016)**.

$$\text{Average body weight} \times \text{vital percentage}$$

$$\text{Production Index} = \text{-----}$$

$$\text{The number of trial day's} \times \text{the food conversion factor} \times 10$$

Table (1):Percentage of components of poultry meat diet

Feed subject	T 0	T1	T2	T3
yellow corn	43	43	42	38.5
Wheat	12	12	12	12
Barley	9	9	9	9
Soy sauce	20	20	17	15
Concentrate	12	11	11	11
Fenugreek	-	1	5	10
Oil	3	3	3	3.5
Limestone	7	7	7	7
Salt	3	3	3	3

Animal protein Golden Jordanian Company contains 50% raw protein, 2200 kilograms of energy represented, 6% fat, 3.5% fiber. Raw, 8 calcium, 3% phosphorous available, 2.75% lysine, 1.8% methionine, 2.3%

methionine + cysteine. **According to the analysis of forage material contained in the reports of the US National Research Council (and NRC 1994).

Statistical analysis

Statistical analysis system **SAS Program (2012)** was used to detect the effect of the difference factors on the study criteria. The LSD test - the least significant difference - was used for a large comparison of means in this study.

RESULTS AND DISCUSSION

Results of this study showed the basic chemical composition of fenugreek seeds such as moisture, protein, fat, ash and carbohydrates for the fenugreek seeds of fenugreek plant and they are 8.90, 24.92, 8.82, 3.08 and 54.28 respectively as in (Table 2).

Table (2): Percentage of the Ingredients of fenugreek seed flour.

Ingredients	(%)
Moisture	8.90
Protein	24.92
Fat	8.82
Ash	3.08
Carbohydrates	54.28

Phytochemical for fenugreek seeds

It was found from the phytochemical detection of the active ingredients of fenugreek seeds with heavy content of tannins, coumarines and flavones, followed by presence of saponins, alkaloids, resins and starch. On the contrary, it was found that the fenugreek seeds are free from the harmful glycosides of living organisms, as in (Table 3). This is consistent with finding by **Dash et al. (2011)** with some differences in quantity, and they found fenugreek seeds free of starch while these study proven presence of starch.

Table (3): Preliminary phytochemical screening of fenugreek seed extract

Phytochemical	Result
Glycosides	-
Saponins	+
Tannins	++
Alkaloids	+
Resins	+
Coumarin	++
Flavones	++
Starch	+

+++ = Present, - = not detected heavy content,

The fatty acids found in fenugreek seeds

The results of the study showed that there are good quantities of the essential fatty acids that are necessary and important for health such as oleic acid, linoleic acid and linolenic, 32.54, 55.04 and 4.55% respectively as in (Table 4). This is confirmed by many other studies that came close to this study **Francis et al. (2002)** and **Wang et al. (2000)**.

Table (4): Percentages of free fatty acids found in fenugreek seed oil

Fatty acids (FA) content	(%)
Arachidonic acid	2.25



Myristic acid	0.072
Palmatic acid	6.95
Stearic acid	2.35
Oleic acid	32.54
Linoleic acid	55.04
Linolenic acid	4.55

Productive characteristics

Effect of fenugreek seed supplementation on average feed consumed by chicken

The results of the study showed a high significant increase ($P < 0.05$) in the average feed consumption for the two treatments T1 and T2 and for the six weeks in a row compared to the control treatment as evident in (Table 5), while the treatment T3 recorded a significant decrease in the average consumption of feed for all six weeks, and this came identical to what he found **Hashemi et al. (2008)**. This indicates that a high concentration of 10% of fenugreek seeds affects negatively and a high moral decline ($P < 0.05$) on the animal, possibly due to the consumption of ground fenugreek seeds that led to a reduction in energy for bio-growth and tissue building by reducing glucose in the blood as explained by **Lima et al. (2017)**; **Abdalla et al. (2011)**, or it may be due to the high content of saponins and steroids with a bitter and pungent taste which negatively affected the leaving of food by the chicks and reduced the appetite of the chicks, which led to a decrease in the rate of feed consumption and a reduction in the resulting weight, and this is confirmed by a study **Jae-Hong et al. (2018)**.

Table (5): Effect of adding different percentages of fenugreek seed flour to animal feed on the rate of feed consumption (gm) by chicks.

Treatment no.	Fenugreek addition (%)	Feed consumption / weeks					
		1 st	2 nd	3 rd	4 th	5 th	6 th
T 0	0	238	438	482	770	740	1137
T1	1	279	409	492	840	819	1139
T2	5	292	389	597	1104	1235	1438
T3	10	225	379	470	882	740	1018
LSD Value*		24.73 *	37.66 *	51.08 *	59.47 *	65.91 *	77.27 *

LSD = ($P \leq 0.05$) *Significant, NS= not significant means

Effect of adding fenugreek seeds to the average body weight of chicken meat

Results of this study showed a significant increase ($P < 0.05$) in all treatments in the average weight of broiler meat for the six weeks as shown clearly in (Table 6). The results showed that the second treatment, T1 and T2 with 1 and 5% of fenugreek addition increased ($P < 0.05$) significantly the average body weight and reached after sixth week to 1994.0 and 1994.0 gm. While, T3 treatments were decreased to 1941 grams compared with control treatment 1958 grams. It is also noted that a significant decrease ($P < 0.05$) in the average body weight, especially in the first two weeks only with the high concentrations of 5 and 10% addition with fenugreek seed, while the high significant increase ($P < 0.05$) returned back with a concentration of 5% and for the remaining weeks it may be due to that small chicks were not bearing the high dose of fenugreek addition and then by the time or perhaps over time the chicks have adapted to the food provided to them. Also, the increase in body weight for the second treatment with 5% fenugreek seed addition, may be attributed to an increase in the level of thyroxin as a result of giving fenugreek seed, which leads to rapid metabolism and thus weight gain, and this is consistent with **Hcin et al. (2018)** and **Ozan et al. (2011)**. The results were consistent with what he mentioned by **Abdouli et al. (2014)** with low concentrations of



fenugreek seeds increased the average weight of the chicks. Moreover, no fatalities were recorded in this study so the reason may be due to the fact that the seeds of the fenugreek contain many vital active ingredients such as essential oils, aldehydes and alkaloids, which increase the body's immunity **Rabia (2010)**. Studies have also demonstrated the effectiveness of the phytochemical components of fenugreek seeds as natural antioxidants for many pathogenic microbes and antioxidants that have increased the body's immunity and which have demonstrated enhanced productive characteristics **Al-Aqil (2016) and Du et al. (2015)**.

Table (6): Effect of adding different percentages of fenugreek seed flour to animal feed on chick's average weight (gm).

Treatment no.	Fenugreek addition (%)	Average body weight (gm) / weeks					
		1 st	2 nd	3 rd	4 th	5 th	6 th
T0	0	196.7	433.4	674.1	1042.0	1384.0	1958.0
T1	1	187.0	422.5	690.8	1075.0	1430.0	1971.0
T2	5	154.2	336.7	625.0	1072.0	1445.0	1994.0
T3	10	137.5	296.7	520.8	899.0	1204.0	1941.0
LSD Value		25.28*	38.66 *	40.28 *	45.71 *	56.90 *	55.02 NS

LSD = (P≤0.05) *Significant, NS= not significant means

Effect of adding fenugreek seed meal to the diet on Weight gain (gm)

The feed conversion factor is one of the basic and necessary indicators to show how efficient the chicks are in representing food and converting it into meat. Thus, the growth and weight of the bodies of bird increases, and any decrease in the value of the food conversion is an indication of increasing its efficiency **El-Shafei et al. (2012)**. The results of the study showed the significant increase observed in (Table 7) in the rate of significant productive increase (P<0.05) for the first and second treatments for all six weeks, while the third treatment with a high concentration of addition 10% led to a high moral decline for all six weeks as well. Therefore, supportive diets with reasonable proportions of fenugreek seeds have contributed to improving the efficiency of utilizing the feed ingested because they contain some stimulants that increase the secretion of growth hormone, which improves the maximum utilization of the feed intake and achieve the increase in weight of the chicks as shown by **El-Shafei et al. (2012)**.

Table (7): Effect of adding different percentages of fenugreek seed flour to animal feed on chick's weight gain (gm).

Treatment no.	Fenugreek addition (%)	Weight gain (gm)/ week					
		1 st	2 nd	3 rd	4 th	5 th	6 th
T0	0	196.7	438.0	240.8	367.9	346.0	525.0
T1	1	129.2	409.0	268.0	384.0	355.0	542.0
T2	5	195.9	389.0	413.0	540.0	633.0	712.0
T3	10	179.0	379.0	306.0	376.7	406.0	517.0
LSD Value		24.08 *	37.66 *	36.74 *	41.07 *	56.82 *	58.94 *

LSD= (P≤0.05) *Significant, NS= not significant means

Effect of fenugreek seed supplementation on average conversion factor

The feed conversion factor is one of the important indicators that show the efficiency of birds converting feed into live weight, and that any decrease in this value is evidence of improved food conversion efficiency. (Table 8), showed a significant decline (P <0.05) of the food conversion factor from the second week to support the diets for the first treatment 1% for fenugreek seeds, and the moral decrease for each of the six weeks for the second treatment

increased by 5% of the addition of fenugreek seeds. And the productivity of the meat required by the consumer. While a significant increase was observed in the feed conversion factor when the fortification ratio increased to 10% of fenugreek seeds and its inverse relationship to weight, weight gain, and productivity of broiler chicks compared to the control treatment. The reason for this is due to the increase in vital substances such as saponins, glycosides, and alkaloids, by increasing the percentage consumed by the chicks that may affect the hormonal system, including lowering the proportion of the hormone insulin which helps to reduce feed consumption **Hashemi et al. (2008)** and this in turn affects the cause of weight reduction and weight productions shown in (Table 8). This is what the consumer rejects and does not meet the need for demand as well as economic losses **Cho et al. (2014)** and **Rabia (2010)**.

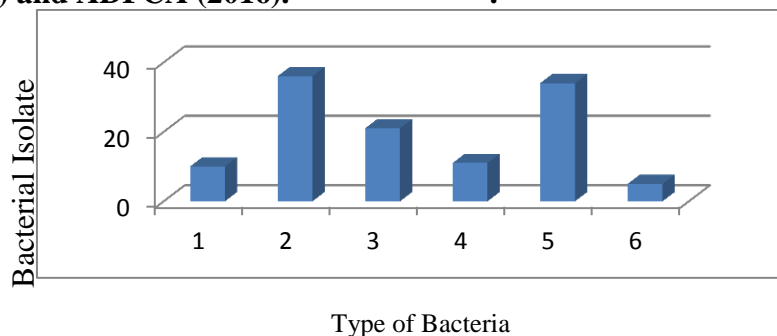
Table (8): Effect of adding different percentages of fenugreek seed flour to animal feed on average conversion factor.

Treatment no.	Fenugreek addition (%)	Efficiency of food transfer / week					
		1 st	2 nd	3 rd	4 th	5 th	6 th
T0	0	1.73	1.83	2.0	2.09	2.14	2.17
T1	1	2.16	1.74	1.83	2.19	1.89	1.75
T2	5	0.976	1.04	1.15	1.33	1.48	1.56
T3	10	1.35	1.95	2.0	2.11	2.14	2.15
LSD Value		0.548 *	0.495 *	0.517 *	0.589 *	0.622 *	0.482 *

LSD = (P≤0.05) *Significant, NS= not significant means

Bacterial content in feeds available in the local market

Numbers of pathological bacterial isolated from poultry feed were showed in figure 1. As it was founded that the number of *Pasteur Ella spp.* bacteria is ten isolates and 36 isolates are from *Diplococcic spp.* and approximately 20 isolates are from *Staphylococcus aurous* and approximately 10 isolates are from *E. coli* bacteria. Almost 32 isolates are *Salmonella spp.* Nearly 4 isolates are *Bacillus spp.* This is conclusive evidence of fodder contamination due to contamination from poultry feces, air and soil. The results were consistent with what was mentioned by **Jae-Hong et al. (2018)**, as it isolated *Bacillus spp.* and *Staphylococcus aureus* bacteria from the environment of poultry fields. Therefore, it is necessary for the feed to be safe, non-contaminated and suitable for animal consumption, as the use of contaminated feed negatively affects the health of the animal and may cause the following: death, miscarriage, reduced production, various disease symptoms such as symptoms of the digestive system, diarrhea, respiratory diseases, and conjunctivitis as well. Proved by many studies and including **Du et al. (2015)** and **ADFCA (2016)**.



1= *Escherichia coli*, 2= *Pasteur Ella spp.*, 3= *Bacillus spp.*, 4= *Staphylococcus aurous* .5= *Salmonella spp*

Figure (1): Numbers of pathogenic bacteria isolated from poultry feed available in the local market.



Sensitivity of the bacterial isolates against antibiotics

The results of the study showed the sensitivity of the studied bacterial isolates with their high sensitivity for all types of antibiotics such as Ampicillin (AMP), Ciprofloxacin (CIP), Erythromycin (E), Nalidexic Acid (NA) and Kanamycin (K) as shown in (Table 9).

Table (9): Bacterial sensitivity to studied antibiotics

+++ =heavy sensitive, ++= medium sensitive, += low sensitive

Bacteria kinds	Sensitivity				
	Ampicillin (AMP)	Ciprofloxacin (CIP)	Erythromycin (E)	Nalidexic Acid (NA)	Kanamycin (K)
<i>E. coli</i>	+++	+	+	+	+
<i>Pasteur Ella</i> spp.	+++	+++	+	++	+
<i>Bacillus</i> spp.	++	++	+	+	+
<i>Staphylococcus Aurous</i>	+++	+++	+	++	+
<i>Salmonella</i>	++	+++	+	+	+

CONCLUSION

The results of the study concluded the importance of strengthening poultry diets with reasonable proportions not exceeding 5% of the fenugreek seeds ground in order to enhance the weight and productivity for the purpose of obtaining a high weight of spawning meat as well as improving the quality of meat and increasing the body's immunity to disease and its resistance to pathogenic microbes that was evident from the absence of any losses In this experiment. We are keen to conduct more research for this ethnic medicinal plant and its positive effects in terms of productivity and weight and its anti-microbial action, and this is due to the superiority of fenugreek seeds by containing high phytochemical compounds that work as antioxidants for many diseases and chronic infections.

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