Iraqi Journal of Agricultural Sciences –2026:57(6):1706-1717
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/rfqn4t66

Effect of Unconventional Peremix on the Productive, Microbial and Histological Traits of Broiler

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ABSTRACT

This study examined the effect of replacing local feed mixture with protein concentrate in broiler feed on production, histological, and microbial characteristics. The study was conducted on 200 one-day-old broiler chicks of Ross 308, randomly divided into four treatments, 50 chicks per treatment, with five replicates and 10 chicks per replicate. The control treatment (T1) was fed 5% protein concentrate, while the protein concentrate in the other treatments (T4, T3, T2) was replaced with protein concentrate in varying proportions (2%, 2.5%, 3%). The feed mixture contains several components, including the carrier materials, which are potato peels at a rate of 33%, and active materials, which are a group of amino acids represented by (methionine, lysine and threonine) and a group of herbs represented by (turmeric, cumin, anise, coriander, cinnamon) in addition to a biological enzyme, a biological antitoxin and probiotic in addition to a mixture of vitamins, minerals and oil. The results showed no significant differences in body weight, feed consumption, weight gain, or feed conversion ratio at the age of (0-39 days). As for the fourth treatment, it led to a significant decrease in the number of bacteria and an increase in villi length. The study concluded that it is possible to replace 3% local feed with 5% imported protein concentrate without negative effects on broiler chickens.

Key words: Amino Acids, Enzyme , Medicinal Herbs, Potato Peels ,Probiotic.

Received: 12/9/2024, Accepted: 22/12/2024, Published: 30/6/2026

INTRODUCTION

The Protein concentrates in broiler feeds constitute 5% of the total materials comprising the feed. This percentage effectively plays a role in breeding success or failure because it ingrates many important nutritional elements in terms of quantity and type, such as amino acids, minerals, vitamins, enzymes, and antioxidants. Any deficiency or imbalance in these elements would negatively affect poultry performance. The protein concentrates used in broiler feed are expensive due to their importation. Their use can lead to several issues, including inconsistent availability in the market, presence of pathogens, difficulty in confirming their type, content, and safety, as well as high protein density in the feed, which can be harmful to the bird's health (Beski et al., 2015). The high protein content in the feed causes a high nitrogen excretion, which leads to an increase in ammonia pollution. To avoid these problems, studies have turned to using amino acids instead of protein concentrates to provide more economical and healthy feed for birds. The amino acids, in particular, Methionine, lysine, threonine, and arginine, are important for improving bird growth. They have a role as antioxidants (Konieczka et al., 2022), improving absorption processes and enhancing immune gene expression in the small intestine (Bhanja et al., 2014; Li et al., 2023). According to the above, global feed manufacturing companies have worked to supplement feed with mixtures called premixes, which are two types of materials: an active material and a carrier material. The active ingredient consists of a mixture of amino acids, vitamins, minerals, enzymes, and antioxidants. The carrier is usually limestone or dicalcium phosphate. The carrier materials

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are a significant financial cost of the pre-prepared mixture. It is necessary to find locally available sources as carrier materials for the manufacture of the feed mixture. Potato peels are one of these materials that provide the feed with nutritional elements. They are a by-product of using potatoes in food in restaurants and homes. Potato peels have a high nutritional value, serving as a source of energy supply at 2043 kilocalories/kg and containing 10.5% protein. The potato peels also contain many active compounds such as tannin acid and chlorophyll, which have an antimicrobial and antioxidant role (Amagloh et al., 2022). The mixture is fortified with a Probiotic and a mixture of some medicinal herbs such as turmeric, coriander, cumin, cinnamon, and anise as a natural growth stimulant and natural antioxidant and a mixture of biological enzymes and a biological antitoxin. The study aimed to using low-priced local sources, such as potato peels and limestone, as carrier materials in the manufacture of feed mixtures. Supplementation with bio-enhance, herbal mixture, biological enzyme mixture and biological antitoxin.Comparing the replacement of protein concentrate with the locally prepared mixture and determining its effect on the productive, microbial, and histological performance of broilers.

MATERIALS AND METHODS

The experiment was conducted in the poultry farm of the Animal Production Department, College of Agricultural Engineering Sciences, University of Baghdad, Abu Ghraib, from 26/12/2023 to 2/3/2024 (39 days). The field experiment comprised 200 one-day-old Ross 308 broiler chicks, and all vaccinations were given to them during the rearing period, which lasted 39 days. The birds were housed in a hall divided by metal wire barriers in the form of pens, each pen measuring (1.7 x 2). The replications were distributed randomly among the pens. The hall was ventilated with fans to extract air, equipped with continuous lighting for 23 hours/day, and water was freely available. The feed was weighed before being provided to the birds and the remaining feed was weighed at the end of each bird's life period to determine the amount of feed consumed at the end of each period. The birds were fed the nutritional treatments (feeds), as shown in Table (1,2,3). The experiment was implemented according to a completely randomized design (CRD), and the means were compared based on Duncan's test. The feed mixture contains several components, including the carrier materials, which are potato peels, at a rate of 33%, and active materials, which are a group of amino acids, represented by (methionine, lysine and threonine) and a group of herbs, represented by (turmeric, cumin, anise, coriander, cinnamon), in addition to a biological enzyme, a biological antitoxin and probiotic, in addition to a mixture of vitamins, minerals and oil , as shown in Table (4). Among the sources of peels, in brief, are the many restaurants, the more waste and the difficulty of disposing of it, in addition to the many cheese factories. Vitamins and minerals are added to ensure that the vitamins in the concentrate and mixture are not damaged during feed manufacturing

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Table 1. Starter feed for broiler chickens for a (0-10) day period ، (Ross 2019)

Feed ingredient%	Treatments			
	T1	T2	T3	T4
Maize	48	45.4	46.1	46.1
Wheat	9.7	10.9	10.5	10.5
Soybean Meal c.p 48%	33	36.8	36.6	36.6
1Protein Concentrate	5	-	-	-
Feed Mix	-	2	2.5	3
Limestone	1.2	0.3	0.2	0.1
Di calcium phosphate	0.7	1.9	1.6	1.6
Oil	2	2.4	2.2	2.3
Salt	0.2	0.3	0.3	0.3
2Vit and minral	0.2	-	-	-
Total	100	100	100	100
3Calculated chemical analysis				
Crude protein %	23.03	23.01	23.00	23.01
Metabolism Energy (Kcal)	3000.00	3006.12	3002.45	3003.70
Methionine %	0.50	0.43	0.44	0.46
Lysine %	1.30	1.30	1.30	1.30
Threonine %	0.87	0.90	0.91	0.92
Calcium %	0.96	0.97	0.95	0.93
Phosphorus %	0.47	0.47	0.42	0.43

1. Protein concentrate type BROCON – 5 SPECIAL W. Each kg contains: 40% crude protein, 5% fat, 2.2% fiber, 4.2% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cysteine, 2.5% sodium, 2107 kcal/kg metabolized energy, 20000 IU vitamin A, 40000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1, 140 mg vitamin B2, 20 mg vitamin B6, 10 mg folic acid, 100 micrograms biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg Iodine, 2 mg Selenium, 6 mg Cobalt, 900 mg Antioxidant (BHG).
2. A mixture of vitamins and minerals, each kg contains: 500 IU Vitamin A, 600 IU D3, 10 mg E, 2 mg K3, 2 mg B1, 2 mg B2, 2 mg B6, 5 micrograms B12, 10 mg C, 15 mg Niacin, 500 micrograms Folic Acid..
3. According to the chemical analysis of the diet onaccording to(NRC,1994).

Table 2. Grower feed for broiler chickens for an)11-24(day period ،(Ross 2019)

Feed ingredient%	Treatments			
	T1	T2	T3	T4
Maize	48	48	47.6	46.8
Wheat	12.8	11.8	11.7	12.1
Soybean Meal c.p 48%	29.1	32.9	32.9	32.8
[1]Protein Concentrate	5			
Feed Mix		2	2.5	3
Limestone	1.1	0.15	0.1	
Di calcium phosphate	0.5	1.65	1.6	1.6
Oil	3.1	3.2	3.3	3.4
Salt	0.2	0.3	0.3	0.3
[2]Vit and minral	0.2			
Total	100	100	100	100
[3]Calculated chemical analysis				
Crude protein %	21.50	21.48	21.49	21.49
Metabolism Energy(Kcal)	3100.00	3098.49	3098.86	3098.99
Methionine%	0.48	0.40	0.42	0.44
Lysine %	1.21	1.20	1.21	1.23
Threonine %	0.80	0.84	0.85	0.86
Calcium %	0.87	0.85	0.90	0.95
Phosphorus %	0.42	0.42	0.41	0.42

1. Protein concentrate type BROCON – 5 SPECIAL W. Each kg contains: 40% crude protein, 5% fat, 2.2% fiber, 4.2% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cysteine, 2.5% sodium, 2107 kcal/kg metabolized energy, 20000 IU vitamin A, 40000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1, 140 mg vitamin B2, 20 mg vitamin B6, 10 mg folic acid, 100 micrograms biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg Iodine, 2 mg Selenium, 6 mg Cobalt, 900 mg Antioxidant (BHG).
2. A mixture of vitamins and minerals, each kg contains: 500 IU Vitamin A, 600 IU D3, 10 mg E, 2 mg K3, 2 mg B1, 2 mg B2, 2 mg B6, 5 micrograms B12, 10 mg C, 15 mg Niacin, 500 micrograms Folic Acid.
3. According to the chemical analysis of the diet onaccording to(NRC,1994).

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Table 3. Finisher feed for broiler chickens for 25-39-day period‹(Ross 2019)

Feed ingredient%	Treatments			
	T1	T2	T3	T4
Maize	48	48.3	48.3	48.3
Wheat	15.6	13.8	13.2	12.7
Soybean Meal c.p 48%	25.3	29.3	29.3	29.3
[1]Protein Concentrate	5	-	-	-
Feed Mix	-	2	2.5	3
Limestone	1	-	-	-
Di calcium phosphate	0.4	1.8	1.7	1.7
Oil	4.3	4.5	4.7	4.7
Salt	0.2	0.3	0.3	0.3
[2]Vit and minral	0.2	-	-	-
Total	100	100	100	100
[3]Calculated chemical analysis				
Crude protein %	20.01	20.00	20.00	20.00
Metabolism Energy(Kcal)	3204.39	3200.1	3207.2	3199.565
Crude Fibers %	0.46	0.4	0.40	0.42
Methionine %	1.11	1.1	1.1	1.1
Lysine %	-	0.78	0.79	0.8
Threonine %	0.80	0.82	0.9	0.9
Calcium %	0.40	0.43	0.42	0.43
Phosphorus %	0.40	0.43	0.42	0.43

1. Protein concentrate type BROCON – 5 SPECIAL W. Each kg contains: 40% crude protein, 5% fat, 2.2% fiber, 4.2% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cysteine, 2.5% sodium, 2107 kcal/kg metabolized energy, 20000 IU vitamin A, 40000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1, 140 mg vitamin B2, 20 mg vitamin B6, 10 mg folic acid, 100 micrograms biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg Iodine, 2 mg Selenium, 6 mg Cobalt, 900 mg Antioxidant (BHG).
2. A mixture of vitamins and minerals, each kg contains: 500 IU Vitamin A, 600 IU D3, 10 mg E, 2 mg K3, 2 mg B1, 2 mg B2, 2 mg B6, 5 micrograms B12, 10 mg C, 15 mg Niacin, 500 micrograms Folic Acid.
3. According to the chemical analysis of the diet onaccording to(NRC,1994).

Table 4. Chemical analysis of the protein concentrate and the mixture prepared locally

Name	Protein center	Feed mixture	Name	Protein center	Feed mixture
1- Metabolism Energy (Kcal/kg feed)	2107	1578.5	8-Threonine %	_	2.59 %
2- Crude protein %	40	12.6 %	9- calcium %	5	16.6 %
3- Ether extract%	5	2.29 %	10- Phosphorus%	4.68	0.85 %
4- Crude Fibers%	2.26	2.00 %	11-Zn(mg/Km)	1.2	1.45
5-Ash%	_	1.09 %	12-VitaminA(IU)	200 000	559 000
6-Methionine %	3.7%	4.06 %	13-Vitamin E (IU)	50	23.6
7-Lysine %	3.85%	3.36 %	14-VitaminC(IU)	_	48.9

Table 5. Active compounds in the locally prepared mixture according to chemical analysis

Name	Feed mixture
Total Phenolic content (mg/100g.)	214.5
Total flavonoid content (mg/100g.)	165.9
Total alkaloid content %	5.6
Total glycoside content %	1.6
Total carotene content %	3.6

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Table 6. Chemical analysis of the raw potato peels

Test Type	Raw potato peels
Protein %	10.5
Ether Extract %	2.3
Moisture %	12.5
Ash %	6.4
Crude Fiber %	20.9
Starch %	10.3
Carbohydrate %	35.4
Similar Energy(kcal)	2043
Carotene %	3.24
Phosphorus %	0.08
Calcium %	0.036
Vitamin A (IU)	3200
Methionine %	0.302
Lysine %	0.442
Cysteine %	0.596
Aflatoxin	UDL

Statistical analysis

The Complete Randomized Design (CRD) was used to investigate the effect of different treatments on the studied traits. The significant differences between the means were compared based on Duncan's (1955) multinomial test. The software SAS was used in the statistical analysis according to the following mathematical model:

$Yij=\mu + Ti + eij$

Where:

Yij: Value of observations due to the treatment.

μ: Grand mean.

Ti: Treatment Effect.

Eij: Random error normally distributed.

Counting the number of E-coli bacteria in the duodenum and ileum

Plate pouring method (Scoters et al., 2000).

Counting the number of Lactobacillus bacteria in the duodenum and ileum

Plate pouring method (Scoters et al., 2000).

RESULTS AND DISCUSSION

Body weight (g): Table (7) shows the effect of total replacement of a locally prepared feed mixture instead of the imported protein (Alwafi) in broiler feed on the average live body weight for all age groups. It is noted in the starter stage at the age of 10 days, a significant superiority at the probability level 0.05 of the control treatment T1 over all treatments. Still, there is no significant difference with T4, the treatment of adding the locally manufactured premix feed mixture at a rate of 3%. There was no significant difference between each of the two treatments (T3 and T4) and (T3 and T2). At the age of 24 days, the results in the same table reference that the control treatment T1 significantly outperformed all treatments containing a feed mixture. At the marketing age of 39 days, it was noticed from the table that there were no significant differences in the average body weight for treatments T3 and T4 compared to the control treatment, while treatment T2 containing a 2% feed mixture recorded a significant decrease (at a significance level P > 0.05) compared to the control treatment T1 and the replacement treatments T3 and T4.

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Table 7. Effect of replacing the imported protein concentrate with the locally prepared feed mixture in the broiler feed on the average live body weight (g) (mean ± standard error) during the different age stages

Treatments	Average live body weight g/bird			
	Initial weight	Weight at age 10 days	Weight at age 24 days	Weight at age 39 days
T1	0.56 ± 41.72 a	15.73 ± 317.7 a	5.157 ± 1107.52 a	89.43 ± 2511.70 a
T2	0.538 ± 41.80	4.11 ± 264.50 c	13.78 ± 860.46 c	32.51 ± 2147.74 b
T3	0.566 ± 42.180	4.47 ± 284.94 bc	9.63 ± 973.38 b	65.71 ± 2411.00 a
T4	0.466 ± 42.02	3.72 ± 291.84 ab	13.31 ± 1005.72 b	54.025 ± 2515.00 a
Significance level	N.S	*	*	*

Significance level / Duncan: * Significant difference (P > 0.05) and **: Significant difference (P > 0.01) Treatments: T1 Control treatment contains 5% protein concentrate (Al-Wafi); T2 Second treatment, Replacement of protein concentrate with feed mixture by 2%; T3 Third treatment, Replacement of protein concentrate with feed mixture by 2.5%; and T4 Fourth treatment, Replacement of protein concentrate with feed mixture by 3%.

Weight gain rate: Table (8) demonstrates that the control treatment T1, using imported protein (Alwafi) at 5%, was superior in the weight gain during the starter and growth stage (from 11 to 24 days) compared to the treatments using a locally manufactured feed mixture (Premix). However, there were no significant differences in weight gain between the replacement treatments of the feed mixture at all percentages and the control treatment T1 during the period from 25 to 39 days. The same table shows that during the total period from 0 to 35 days, the differences for the two treatments of replacing the feed mixture (T3 and T4) with the protein concentrate were insignificant compared to the comparison treatment T1, while the treatment T2, containing the feed mixture at a 2%, recorded a significant decrease (P > 0.05) compared to the control treatment T1 and the other two replacement treatments T3 and T4.

Table 8. Effect of replacing locally prepared feed mixture (Premix) instead of imported protein (Al-Wafi) to the broiler feed on the rate of weight gain of the live body (g) (mean ± standard error) during the different age stages

Treatments	Weight gain rate			
	0-10 days	11-24 days	25-39 days	0-39 days
T1	15.97 ± 275.98 a	12.29 ± 789.82 a	90.35 ± 1404.18 ab	89.88 ± 2469.98 a
T2	3.863 ± 222.70 b	12.86 ± 595.96 c	26.69 ± 1287.28 b	32.50 ± 2105.94 b
T3	4.47 ± 242.76 b	10.86 ± 688.44 b	63.34 ± 1437.62 ab	66.04 ± 2368.82 a
T4	3.41 ± 249.82 b	13.58 ± 713.88 b	43.78 ± 1509.28 a	53.80 ± 2472.98 a
Significance level	*	*	*	*

Significance level / Duncan : * Significant difference (P > 0.05) and **: Significant difference (P > 0.01) Treatments: T1 Control treatment contains 5% protein concentrate (Al-Wafi); T2 Second treatment, Replacement of protein concentrate with feed mixture by 2%; T3 Third treatment, Replacement of protein concentrate with feed mixture by 2.5%; and T4 Fourth treatment, Replacement of protein concentrate with feed mixture by 3%

Feed consumption rate: The results in Table (9) show significant differences in feed consumption rates between the treatments across all periods, with a probability level of 10.0 > P. Treatment T2 showed a significant decrease in feed consumption from 0 to 10 days compared to the control treatment T1, while treatments T3 and T4 did not exhibit

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significant differences compared to T1. During the two growth and final periods (11-24 and 25-39 days), the same effect appeared of a significant decrease in treatment T2, in which a 2% feed mixture was used, compared to the control treatment T1 and the other treatments T3 and T4, as the rate of feed consumption did not differ among them. Concerning the cumulative feed quantity during the period from 0 to 39 days, the differences were insignificant between the two treatments of replacing the feed mixture by 2.5 and 3% for treatments T3 and T4 compared to the control treatment T1, while all of these treatments were superior to the replacement treatment T2 containing the mixture by 2%.

Table 9. Effect of adding locally prepared feed mixture (Premix) instead of imported protein (Al-Wafi) to broiler feed on the rate of feed consumed (g/bird) for broiler chickens (mean ± standard error) during the different age stages.

Treatments	Feed consumption rate			
	0-10 days	11-24 days	25- 39days	Cumulative feed (Experiment end)
	ab	a	a	a
T1	4.77 ± 274.62	21.00 ± 1211.12	24.165 ± 2463.70	30.06 ± 3949.44
	c	b	b	c
T2	4.93 ± 238.66	22.46 ± 1045.76	46.61 ±2181.74	55.78 ± 3466.16
	b	a	a	a
T3	5.39 ± 260.58	27.04 ± 1174.68	32.14 ± 2461.40	47.53 ± 3896.66
	a	b	a	b
T4	6.00 ± 288.32	28.56 ± 1182.96	23.64 ± 2461.60	45.64 ± 3932.88
Significance level	*	*	*	*

Significance level / Duncan : * Significant difference (P > 0.05) and **: Significant difference (P > 0.01)
Treatments: T1 Control treatment contains 5% protein concentrate (Al-Wafi); T2 Second treatment, Replacement of protein concentrate with feed mixture by 2%; T3 Third treatment, Replacement of protein concentrate with feed mixture by 2.5%; and T4 Fourth treatment, Replacement of protein concentrate with feed mixture by 3%.

Feed conversion ratio: The results in Table (10) refer to insignificant differences between treatments T2 and T3 compared to treatment T1, while treatment T4 recorded a significant deterioration compared to treatment T1 during the period 0-10 days. During the period from 11 to 24 days, treatments T2 and T3 recorded a significant deterioration (P > 0.05) compared to T1 and T4, while treatment T4 did not differ significantly from the control treatment T1. Regarding the period from 25 to 39 days and the total period from 0 to 39 days, there were no significant differences between the treatments of replacing the local feed mixture compared to the control treatment T1. The absence of significant differences in the productive performance (live body weight, weight gain, and feed conversion ratio) of the treatments, especially T3 and T4, in comparison with the control may be due to the combination of the mixture consisting of potato peels, herbal mixture, Probiotic, as well as biological antitoxins and biological enzymes. The combination of these ingredients enhances the productive performance of broiler chickens. They act as natural antioxidants and growth stimulants, improving digestion and absorption, which helps increase food utilization. The mixture contains several active compounds, such as Phenol, Flavonoid, alkaloid, glycoside, and carotene (Table 4). These compounds, especially phenols and flavonoids, play an important role as antioxidants, protecting body cells and muscle tissues against oxidation (Predescu et al., 2024). These compounds also play a positive role in improving poultry performance through their effective role in improving food digestion and utilization, in addition to their role as antibacterial agents that reduce harmful bacteria (Milan et al., 2008). The inclusion of phenolic compounds in bird feed promotes the health of the intestinal flora, which in turn boosts the efficiency of digestive enzymes and, ultimately, the efficiency of nutrient digestion . These active compounds may enhance the characteristics of the villi by increasing the length and thickness of the mucous layer (Such et al., 2021) , as shown in Table 7. This improvement can lead to better

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absorption and utilization of nutrients, resulting in improved performance in birds. Alternatively, perhaps the absence of negative effects when using the mixture at 2.5 and 3% may be due to the role of the added enzyme mixture, as enzymes play an important role in improving the poultry's productive performance by improving the nutritional value as well as increasing the digestion of nutrients, which is positively reflected in the performance of broilers, and this was confirmed by Al-Mashhadani (2018) when using an enzyme in broiler feed. The effectiveness of the mixture may be due to the active ingredient in potato peels. The peels contain various active compounds such as phenolic compounds and flavonoids, as well as the phytase enzyme (Dvořáková, 1998). The phytase enzyme is important as it helps break down proteins into amino acids, increases their absorption in the intestines, and aids in releasing protein and starch from phytate (Rostami & Giri, 2013). Medicinal herbs play an important role in improving the productive performance of poultry because they contain effective compounds, especially the mixture used in the study (turmeric, cumin, coriander, cinnamon, and anise). Yang et al. (2021) indicated that the presence of these herbs in broiler feed improves productive performance.

Table 10. Effect of adding locally prepared feed mixture (Premix) instead of imported protein (Alwafi) to broiler feed on the feed conversion ratio (g/g weight gain) for broiler chickens (mean ± standard error) during the different age stages.

	Food conversion ratio			
Treatments	**0-10 days**	**11-24 days**	**25-39 days**	**0 -39 days**
T1	b 0.05 ± 1.00	b 0.032 ± 1.53	0.12 ± 1.78	0.07 ± 1.609
T2	ab 0.01 ±1.07	a 0.037 ± 1.756	0.02 ± 1.695	0.015 ± 1.646
T3	ab 0.012 ± 1.07	a 0.059 ± 1.709	0.09 ± 1.728	0.061 ± 1.651
T4	a 0.01 ±1.15	b 0.025 ± 1.516	0.01 ± 1.568	0.020 ± 1.51
Significance level	*	*	N.S	N.S

Significance level / Duncan : * Significant difference (P > 0.05) and **: Significant difference (P > 0.01) Treatments: T1 Control treatment contains 5% protein concentrate (Al-Wafi); T2 Second treatment, Replacement of protein concentrate with feed mixture by 2%; T3 Third treatment, Replacement of protein concentrate with feed mixture by 2.5%; and T4 Fourth treatment, Replacement of protein concentrate with feed mixture by 3%

Morphological changes in the duodenal portion of the small intestine: Data listed in Table (11) indicates. The effect of replacing the protein concentrate in broiler feeds with the locally prepared mixture on some histological traits of the duodenal region (villus height, crypt depth, and mucosal layer thickness) indicate that there was a significant improvement in the height of the villi in the treatments T3 and T4 when the percentage of using the feed mixture increased to 2.5 and 3%, respectively, as these two treatments recorded the highest height of the villi. Treatment T4 recorded a significant superiority (P > 0.05) in the depth of the crypts compared to the control treatment and the other treatments, but treatment T3 recorded the same result compared to T1 and T2. The study found that treatment T4 did not show a significant difference in villus thickness compared to T1. However, treatments T2 and T3 showed a significant decrease in villus thickness compared to T1 and T4, suggesting that increasing the use of the feed mixture improved most of the morphological traits of the duodenum. The increase in villus length and crypt depth in replacement treatments T3 and T4 may be attributed to the higher levels of active compounds in the mixture, such as phenols and flavonoids (refer to Table 5). These compounds make the digestive tract more digestible and absorbable by stimulating the division of epithelial cells lining the intestine. This process acts as an antibiotic to suppress germs and helps improve the digestion process (Lee et al., 2020). The

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P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/rfqn4t66

presence of microorganisms in the mixture, along with the combination of herbs containing multiple sugars, might act as a nutritional medium for the growth of beneficial microorganisms, which helps to maintain a balanced microorganism population in the intestines. The microorganisms produce short-chain fatty acids that stimulate the differentiation of the epithelial cells lining the digestive tract by providing them with energy to function continuously. This stimulation leads to an increase in the length of the villi, which aids in the digestion and absorption of food (Chiang et al., 2009).. The increased thickness of the villi may be attributed to the active compounds present in the herbal mixture, such as phenols, flavonoids, carvacrol, quercetin, and thymol. These compounds enhance the efficiency of the digestive tract by promoting division in the epithelial cells lining the intestines and boosting antibiotics that suppress germs, thus improving the digestion process. Additionally, herbs may contain substances like Fructooligosaccharides (FOS) or Polysaccharides, which reduce the diameter of the intestine, slowing the passage of food and, hence, enhancing digestion and absorption (Lee et al., 2020).

Table 11. Effect of adding locally (Premix) instead of imported protein (Al-Wafi) to broiler diets on the length of the villus, its thickness, and the depth of the crypts in the duodenum parts of the small intestine of broiler at the age of 39 days (mean ± standard error).

Treatments	Duodenum crypt depth	Duodenal villi thickness	Duodenal villi height
T1	c 4.27 ± 91.30	a 5.13 ± 84.33	B 23.33 ± 432.21
T2	c 1.40 ± 88.86	b 3.19 ± 60.84	C 4.56 ± 297.80
T3	b 0.68 ± 118.24	b 1.93 ± 58.86	A 36.95 ± 504.63
T4	a 1.18 ± 132.68	a 4.08 ± 73.82	A 12.41 ± 522.90
Significance level	*	*	*

Significance level / Duncan : * Significant difference (P > 0.05) and **: Significant difference (P > 0.01)Treatments: T1 Control treatment contains 5% protein concentrate (Al-Wafi); T2 Second treatment, Replacement of protein concentrate with feed mixture by 2%; T3 Third treatment, Replacement of protein concentrate with feed mixture by 2.5%; and T4 Fourth treatment, Replacement of protein concentrate with feed mixture by 3%.

Microorganism: Table (12) compares the treatments adding the feed mixture (Premix) with the control treatment to which imported protein (Al-Wafi) was added. There are no significant differences between all treatments regarding the number of beneficial bacteria (Lactobacillus). As for harmful bacteria (*E. Coli*), the treatment of addiction T4 containing the locally prepared feed mixture (Premix) by 3% was superior to all treatments for containing the lowest number of harmful bacteria (*E. Coli*). Regarding the (Total Count), the total number of microorganisms, the T4 treatment containing the feed mixture (Premix) by 3% was superior to all treatments, as it contained the lowest number of total microorganisms. The decrease in the number of harmful bacteria in treatment T4 (by increasing the replacement percentage) may be due to the medicinal herbs and their content, which play a role in inhibiting the number of harmful microorganisms. These herbs have some effective compounds, such as cinnamaldehyde, carvacrol, and quercetin, which work as antidotes to harmful microorganisms and maintain beneficial bacteria in the digestive system. These substances act as antimicrobial agents and protect beneficial bacteria in the digestive system. These compounds affect the function of bacterial cell membranes, especially those of *E. coli* and *Salmonella*. The higher the replacement rate in the feed mixture, the higher the percentage of active compounds that show an effective effect on harmful microorganisms. This is what happened in Table 11, especially the T4 treatment because it has the highest replacement rate for the feed

Iraqi Journal of Agricultural Sciences –2026:57(6):1706-1717
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/rfqn4t66

mixture. They also stimulate the intestines to secrete mucus, which in turn reduces the adhesion of harmful bacteria to the dorsal cells of the digestive tract , The change in *E.coli* levels during the use of the feed mixture containing the probiotic and the herbal mixture is due to the change in pH, which makes the medium acidic, and this medium affects the *E. coli* bacteria. (Jamroz et al., 2006).

Table 12. Effect of adding locally prepared feed mixture (Premix) instead of imported protein (Al-Wafi) to broiler feed on the numbers of harmful and beneficial microorganisms in the small intestine of broiler carcasses at the age of 39 days (mean ± standard error).

E. Coli	lactic acid bacteria	Treatments
a 0.27±7.52	0.35±8.23	T1
a 0.42±7.72	0.25±9.19	T2
a 0.01±7.95	0.08±9.19	T3
b 0.41±6.16	0.53±9.06	T4
*	N.S	Significance level

Significance level/ Duncan: N.S insignificant, * Significant difference (P < 0.05) and **: Significant difference (P < 0.01)Treatments: T1 Control treatment contains 5% protein concentrate (Al-Wafi); T2 Second treatment, Replacement of protein concentrate with feed mixture by 2%; T3 Third treatment, Replacement of protein concentrate with feed mixture by 2.5%; and T4 Fourth treatment, Replacement of protein concentrate with feed mixture by 3%

CONCLUSION

Based on our study of adding different proportions of locally prepared feed mixture (Premix) to broiler feed over the experimental period of 0-39 days, we conclude that using 2.5% and 3% proportions did not have a negative effect, even with a high replacement percentage. The results were similar to the control treatment. Additionally, we observed an improvement in the small intestine environment, with a decrease in harmful bacteria and total microorganisms in the duodenum tissue section for the T4 treatment, which had the best feed mixture (Premix) proportion of 3%, which led to significant trait improvements.

ACKNOWLEDGEMENT

The authors would like to thank the University of Baghdad for supporting this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

Funds: this research received no funds.

AUTHOR'S CONTRIBUTION STATEMENT

Ahmed K.H. Hussein, a master's student, wrote this article from his thesis; H . A . Al – Mashhadani, supervisor.

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DOI: https://doi.org/10.36103/rfqn4t66

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تأثير مخلوط علفي غير تقليدي في الصفات الانتاجية والميكروبية والنسيجية لدى فروج اللحم

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المستخلص

تناولت الدراسة تأثير استبدال مخلوط علفي محضر محلياً بالمركز البروتيني في علائق فروج اللحم في الاداء الانتاجي وبعض الصفات النسيجية والميكروبية للأمعاء . أجريت الدراسة على 200 فرخ من فروج اللحم التجاري روز 308 بعمر يوم واحد، قسمت الافراخ عشوائياً إلى أربع معاملات بواقع 50 فرخ لكل معاملة وتضمنت المعاملة الواحدة 5 مكررات إذ تعد T1 معاملة مقارنة تحتوي على علائق فيها مركز بروتيني مستورد بنسبة 5% اما المعاملات T4،T3،T2 إذ تم فيها إحلال المخلوط العلفي بنسبة 2% ،2.5% ،3% على التوالي .وإن الخليط العلفي المستعمل مكون من عدة مواد متمثلة بالمادة الحاملة وهي قشور البطاطا إذ تشكل 33% من مكونات الخليط علاوة على مواد مثل الاحماض الامينية (المثيونين ، اللايسين والثريونين) ومجموعة اعشاب طبية (كركم ، كمون، يانسون ، كزبرة ، دارسين) بالإضافة الى انزيم بايلوجي ومضاد سموم بايلوجي ومعزز حيوي وخليط من الفيتامينات والمعادن والزيت . أظهرت النتائج عدم وجود فروف معنوية في وزن الجسم الحي ومعدلات استهلاك العلف ومعدل الزيادة الوزنية ومعامل التحويل الغذائي بين المعاملات عند عمر التسويق (39 يوم) . كما أظهرت المعاملة الرابعة انخفاضاً في اعداد بكتريا القولون وزيادة في طول الزغابات .ومن الدراسة الحالية يمكن الاستنتاج بإمكانية استبدال المخلوط العلفي المحلي وبالنسبة 3% محل المركز البروتيني المستورد من دون آثار سلبية على فروج اللحم .

الكلمات المفتاحية: الأحماض الأمينية، الإنزيمات، الأعشاب الطبية، قشور البطاطس، المعزز الحيوي.