The effect of substituting local de-hulled sunflower meal instead of soybean meal with or without the addition of the probiotic on the productive and physiological performance of broiler chicks.

Mohammed I. ALmolah [1] **Ibrahim S. Kloor [2]**

Department of Animal Production, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq 1,2

ABSTRACT

This study was conducted in the Poultry Research farm of the Department of Animal Production, College of Agriculture and Forestry/University of Mosul,with the aim of studying the effect of replacing local de-hulled sunflower meal with soybean meal with or without the addition of the probiotic on the productive and physiological performance of broiler chickens. To do this,a total of 360 unsexed (Ross-308) one-day-old broiler chicks were used, randomly distributed in a two-factor experiment (4×2) into eight feeding treatments, in each treatment three replications, a rate of 15 chicks for each replicator, homogeneous in weight, and for a rearing period that lasted 42 days of age, during which they were fed at four levels (0,33,66, and 100%) of de-hulled sunflower meal protein with or without the addition of the probiotic. The results showed that there were no significant differences between the control treatment and the partial replacement treatment (33% Dh-SFM) in live body weight, feed conversion factor, carcass characteristics, microbial content, or gut morphology. while increasing the level of substitution to 66 and 100% led to a significant deterioration($P \leq 0.05$). in all these traits. The results of the interaction showed that the partial replacement treatment (33% Dh-SFM with the probiotic) was significantly superior ($P \leq 0.05$) to the negative control treatment in live body weight, weight gains, production index, intestinal content of beneficial bacteria (*Lactobacillus*), and villus height. While the results showed significant deterioration in the partial and total substitution treatments (66 and 100%Dh-SFM with or without probiotic) in all these traits**.**

Keywords: De-hulled sunflower meal, Soybean meal, Probiotic, Broiler.

INTRODUCTION

Providing human food in a sustainable manner is one of the main challenges facing the world in the twenty-first century [15]. Poultry farming, as the most sustainable livestock sector, contributes significantly to ensuring the country's food security. The demand for poultry meat products has increased over the decades. The latter comes with an increase in awareness of its nutritional value and lower prices compared to red meat products. Poultry meat is characterized by its high protein content and low-fat content, which makes it a healthy and therapeutic food. In order to meet the increasing demand for poultry meat, it has become necessary to improve the efficiency of feeding broilers [20] by equipping them with energy and protein sources that ensure their growth in the best possible way, and attention to the nutritional aspect in any production process is a major focus. In determining the success or failure of the production process, as it constitutes 60–70% of the costs of meat production, the feed industry used in feeding poultry relies on the use of soybean meal as a protein source in diets, which has led to an increase in demand for it and an increase in its prices locally [2]

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Therefore, the search for alternative local sources of imported vegetable protein has become of interest to researchers and nutrition experts. Sunflower meal (SFM), the by-product of the process of extracting oil from sunflower seeds, is one of the protein sources commonly used in many countries of the world in animal and poultry feed due to the cheapness of its protein unit compared to soybean meal [19]. Sunflower meal contains a protein content ranging from 24–48%, depending on its content of dietary fiber (8–36%), due to the inverse relationship between them [25]. Despite this, the use of sunflower meal in poultry feed is limited due to its high content of dietary fiber (non-starchy polysaccharides) that include cellulose, hemicellulose (xylan), and lignin, which act as anti-nutritional agents, negatively affecting the energy content and amino acids in sunflower meal [26]. Many attempts have been made to improve the nutritional value of sunflower meal, such as increasing and improving the process of removing husks, adding external enzymes and probiotics [3], and the use of probiotics in broiler diets is one of the methods used to improve the efficiency of digestion. and the absorption of nutrients in the gastrointestinal tract [16], where studies have shown the ability of probiotics to create a microbial balance in the digestive tract [28], increase the numbers of beneficial bacteria, inhibit the growth of pathogenic bacteria by the competitive or exclusionary action possessed by the probiotic [21], and boost vital immunity. In addition to the ability of the microorganisms in the probiotic and beneficial bacteria to secrete digestive enzymes that support the work of internal enzymes in the digestion of nutrients [32]. Accordingly, this study aimed to improve the nutritional value of de-hulled sunflower meal by treating them with the addition of a probiotic and knowing their effect on the productive and physiological performance of broiler chickens.

MATERIALS AND METHOD

The location and purpose of the experiment

This study was conducted in the Poultry Research farm of the Department of Animal Production, the College of Agriculture and Forestry, and the University of Mosul for the period from 11/3/2022 to 1/12/2022, with the aim of studying the effect of replacing local de-hulled sunflower meal with soybean meal with or without the addition of a probiotic on the productive and physiological performance of broiler chickens.

Experiment chicks:

In this study, a total of 360 unsexed one-day-old broiler chicks (Ross-308) were used, and their average weight at hatching was (41.5) grams. This weight was adopted as a starting weight for experimental treatments. The chicks were randomly divided in a factorial experiment with two factors (4×2) to eight feeding treatments, with three replications for each treatment and 15 chicks for each replicate, homogeneous in their weights. And for a rearing period that lasted 42 days.

Experimental diets:

The use of de-hulled sunflower meal (Dh-SFM) in the diet of this study as a partial and total substitute for soybean meal at levels (0, 33, 66 and 100%) in experimental diets with or without the addition of probiotics, and the adult rearing period was divided (42) days into two stages, and two rations of different replacement levels were formed for both periods (Table 1), the first being the starting ration for the period from one to 21 days, then it was replaced by a finished ration that was used to feed the broilers for the period from 22 to 42 days. In the formation of these empirical relationships, he relied on the recommendations of the US National Research Council [25]. The process of substituting de-hulled sunflower meal instead of soybean meal was carried out on the basis of nitrogen symmetry (iso-nitrogenous) and represented energy (iso-caloric), meaning that the substitution was calculated on the based on of protein/protein for both meals. The commercial probiotic mixture consisting of Bacillus Subtilis-PB6 at a concentration of (50,000×10^7) CFU/kg, and Enterococcus faecium (DSM 7134) at a concentration of (1,500×10^9) CFU/kg was used, where the probiotic mixture was added to the diet in an amount of 400g/ton of feed according to the manufacturer's instructions. Feed and water were given to the chicks freely, ad-libitum, throughout the rearing period.

Table (1) Dietary ingredients and chemical composition of starter and finisher diets experiment.

Ingredients	Diets							
	Starter (1-21 day)				Finisher (22-42 day)			
	0 % Dh-SFM	33% Dh-SFM	66% Dh-SFM	100% Dh-SFM	0 % Dh-SFM	33% Dh-SFM	66% Dh-SFM	100% Dh-SFM
Soybean Meal	37	24.75	12.25	0	31	20.50	10.25	0
Dh-SFM	0	11.25	22.5	33.75	0	9.5	19	28.25
Corn	36	37	38.25	40.25	48	49	49.75	51.75
Wheat	21	21	21	20	15	15	15	14
Soybean Oil	3	3	3	3	3	3	3	3
Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lime stone	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Actual Chemical Analysis (%)								
Moisture	10.62	10.41	10.2	10.05	10.63	10.56	10.48	10.36
Crude Protein	23.12	23.14	23.17	23.2	20.55	20.5	20.64	20.59
Crude Fiber	3.91	5.29	6.98	8.71	3.88	5.22	6.35	7.96
Ether Extract	4.02	4.63	4.93	5.45	4.25	4.88	5.01	5.33
Crude Ash	4.85	4.82	4.77	4.62	4.54	4.45	4.39	4.3
Calculated Chemical Analysis **								
M.E(Kcal/Kg)	3042	3038	3037	3038	3130	3128	3124	3127
Lysine %	1.47	1.25	1.02	0.8	1.3	1.11	0.92	0.74
Methionine %	0.59	0.6	0.61	0.62	0.56	0.57	0.57	0.58

-The amount of energy represented and the percentage, amino acids were calculated from the tables of chemical analysis [25] and according to the proportions of raw materials in the diets, **Dh-SFM is de-hulled sunflower meal, **M.E** is Metabolic energy.

Studied traits:

The growth and carcass characteristics of all treatments were studied during the rearing period of 42, where the live body weight and the amount of feed consumed were measured weekly, and the weekly weight gains and feed conversion factor were calculated, as per the Production Index (PI) and the European Productive Efficiency Factor (EPEA), at the end of the experiment period, (6) birds were selected from each treatment,

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and their weight was recorded to represent the live weight at marketing. The relative weight of the ingested internal organs (liver, heart, and gizzard) and belly fat was also measured, and the ratio of their weights to the weight of the final living body. One gram of intestinal contents (jejunum, ileum, and two caecum) was taken for each bird and placed in special tubes for the purpose of conducting the transplant process and estimating the microbial content in it. Then, 1 cm samples were taken from the middle of the small intestine near Meckel's diverticulum at the end of the jejunum. (Jejunum) to measure the morphological characteristics of the tissue of the jejunal segment of the small intestine.

Statistical analysis:

The data of the experiment were analyzed statistically using a complete randomized design (C.R.D.) for a factorial experiment with two factors (4×2), and the significance of the differences was tested using Duncan's multinomial test [8], at a probability level ($p \leq 0.05$). The statistical analysis of the data was performed using the ready-made statistical analysis program [27].

RESULTS AND DISCUSSION

Growth performance:

Table (2) shows that there were no significant differences in the final live weight and total weight gains between the control treatment (0% Dh-SFM) and the second treatment in which de-hulled sunflower meal was used (33%) as a protein source instead of soybean meal. While a significant ($P \leq 0.05$) and linear decrease was observed in both traits, with an increase in the levels of substitution of de-hulled sunflower meal to 66 and 100% (the third and fourth treatments) compared with the control treatment. As for the effect of the interaction between the replacement of de-hulled sunflower meal instead of soybean meal and the addition of the probiotic, The results (Table 2) indicate a significant improvement ($p \leq 0.05$) in the final live weight and total weight increases for the case of interaction between the addition of the probiotic and all levels of replacement of de-hulled sunflower meal (33, 66 and 100% Dh-SFM) compared with the same The corresponding level without addition. When comparing all substitution treatments, whether with or to which the probiotic was added, with the negative and positive control treatments, we notice a significant decrease ($p \leq 0.05$) for all cases of overlap between the 66 and 100% substitution levels and the probiotic compared with the negative and positive control treatments. These results agreed with [4,9], which showed that the weights of broilers were not affected by replacing sunflower meal with soybean meal by 10–30%, and they noticed a significant decrease ($p \leq 0.05$) with an increase in the percentage of replacement in the bush. The reason for the decrease in growth performance when replacing de-hulled sunflower meal by 66 and 100% may be attributed to the increase in the level of non-starch polysaccharides (NSP) in these diets, as NSP works to lock nutrients inside the cell wall and make them unavailable for the action of digestive enzymes, in addition to reducing the transit time of the food mass (Digesta) inside the gastrointestinal tract [7], and thus increasing the amount of undigested nutrients excreted outside the body, which reduces feeding efficiency. On the other hand, the reason for the improvement in growth performance as a result of the addition of the probiotic compared to the treatments devoid of it may be due to the role of the bacteria that make it up in improving the nutritional value of de-hulled sunflower meal and the whole diet through its secretion of digestive enzymes, where Bacillus spp. works to secrete the xylanase enzyme that reduces One of the negative effects caused by non-starch polysaccharides is the degradation of xylan and arabinoxylan sugars, which leads to a decrease in viscosity and the release of retained nutrients [13,6].

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Table (2) Effect of substituting de-hulled sunflower meal as a substitute for soybean meal with or without the Probiotic on the growth performance of broiler chickens.

Treatments	Initial body weight (g/bird)	Final body weight – 6th week (g)	Total weight gain (g/bird)
Level of Dh-SFM			
Control	41.51±0.01	2861.90±37.24 a	2820.38±37.23 a
33 % Dh-SFM	41.44±0.02	2838.13±30.19 a	2796.68±30.19 a
66 % Dh-SFM	41.52±0.01	2208.00±45.01 b	2166.48±45.01 b
100 % Dh-SFM	41.54±0.01	1956.53±27.41 c	1914.98±30.32 c
Probiotic			
W.O Probiotic	41.50±0.01	2399.10±118.3 b	2357.60±118.38 b
With Probiotic	41.52±0.01	2533.18±121.96 a	2491.65±120.64 a
Dh-SFM × Probiotic			
Control (-) W.O Probiotic	41.49±0.01	2788.70±16.48 b	2747.21±16.48 b
Control (+) With Probiotic	41.55±0.02	2935.10±36.14 a	2893.55±36.17 a
33 % Dh-SFM W.O Probiotic	41.44±0.01	2782.00±35.98 b	2740.56±35.98 b
33 % Dh-SFM With Probiotic	41.46±0.01	2894.25±10.62 a	2852.79±10.63 a
66 % Dh-SFM W.O Probiotic	41.53±0.01	2136.00±50.72 d	2094.48±50.77 d
66 % Dh-SFM With Probiotic	41.52±0.01	2280.00±48.68 c	2238.48±48.66 c
100 % Dh-SFM W.O Probiotic	41.53±0.01	1889.70±40.30 f	1848.17±40.31 f
100 % Dh-SFM With Probiotic	41.55±0.02	2023.35±20.61 e	e1981.80±24.84

Different letters in the column represent significant differences at the probability level (p≤0.05). **Dh-SFM** is de-hulled sunflower meal; **W.O** is without.

Feed Consumption and Feed Conversion Factor:

The results in Table (3) show that there was a significant decrease (P≤0.05) in the amount of total consumed feed and the feed conversion factor as a result of substituting partially and completely de-hulled sunflower meal at a ratio of 33, 66, and 100% Dh-SFM to replace soybean meal in the second treatment. The third and fourth, respectively, were compared with the control treatment. As for the effect of adding bio-enhancement to the diet. A significant increase (P≤0.05) was observed in the total feed consumption by birds as a result of the addition of the probiotic compared to the treatment without the addition, while the feed conversion factor was not affected by the addition of the probiotic. As for the effect of the interaction between the replacement of de-hulled sunflower meal with soybean meal and the addition of the probiotic, it is clear from the results that the presence of the probiotic in the control

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treatment and the replacement treatments of de-hulled sunflower meal at levels 33, 66, and 100%, increased significantly ($p \leq 0.05$) of their feed consumption compared with the same corresponding level without addition, except for the partial replacement treatment (33%), in which their feed consumption was not affected by the addition of the probiotic. It is also noted from the results that the probiotic did not significantly affect the feed conversion factor when added to the control treatment and the replacement treatments of de-hulled sunflower meal at levels (33, 66, and 100%), compared with the corresponding level without addition. These results are in agreement with [2,31], who noticed a significant decrease ($p \leq 0.05$) in the amount of feed consumed and a deterioration in the feed conversion factor when replacing sunflower meal (25-75%) with soybean meal in diets. Broilers compared with the control treatment. The reason for the decrease in the amount of feed consumed and the deterioration of the feed conversion factor with the increase in the replacement of de-hulled sunflower meal instead of soybean meal by 66 and 100% may be due to the increase in the proportion of non-starch polysaccharides. As mentioned by [30], the physical density of the feed changes depending on the level of fiber in sunflower meal, which leads to a reduction in feed consumption due to the large volume occupied by fiber in the digestive system. On the other hand, the reason for the improvement in the amount of feed consumed when adding the probiotic is attributed to the improvement of the coefficient of digestion of nutrients through the enzymes produced by the bacteria included in its composition, especially the enzymes that analyze non-starchy polysaccharides (NSPase) in sunflower meal and the diet as a whole and reduce their negative effects, improving the gut environment [18]. On the other hand, [1] confirmed that the probiotic works to reduce the time of gastric emptying, which leads to an increase in feed consumption.

Productive Performance Indicators:

Table (3) shows that there were no significant differences between all treatments in the percentage of Mortality, and the results showed that the second treatment (33% Dh-SFM) matched the control treatment in the values of the European Productivity Index and the European Productive Efficiency Factor, while a significant decrease was observed ($p \leq 0.05$) and linearly in these parameters with increasing levels of de-hulled sunflower meal substitution to 66 and 100% compared with the control treatment. As for the effect of the interaction between the replacement of de-hulled sunflower meal instead of soybean meal and the addition of the probiotic, The results in Table (3) indicate that the production index scale and the European production efficiency factor were not affected by the addition of the probiotic to the substitution coefficients for de-hulled sunflower meal at levels (33, 66, and 100%), compared with the corresponding level without addition. This result agreed with the results of [22,23] that the productive performance of broilers was not affected when sunflower meal was replaced by (5–20%) soybean meal instead of soybean meal. The reason for the improvement in productive performance when adding the probiotic may be attributed to the beneficial effect of the probiotic on the health of the bird through its direct nutritional action as bio-regulators of the intestinal microflora and strengthening the body's natural defense function [12]. It is also considered an alternative to antibiotics and growth stimulants. This is because it has a competitive or exclusionary effect on pathogenic microflora, thus stimulating microbial balance, changing the type and number of gut microflora, and increasing the body's resistance to disease [11].

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Table (3) Effect of substituting de-hulled sunflower meal as a substitute for soybean meal with or without the Probiotic in feed consumption, feed conversion factor, and Productive Performance Indicators of broiler chickens (means ± standard error).

Treatments	Total Feed Consumed (g)	Total Feed Conversion Factor (g)	Mortality (%)	Productive Index (PI)	(EPEF)
Level of Dh-SFM					
Control	4345.41±24.81 a	1.54±0.01 b	1.11±1.11	437.83±11.42 a	439.86±15.31 a
33 % Dh-SFM	4200.39±25.38 b	1.50±0.009 b	0.00±0.00	450.00±7.36 a	456.67±7.39 a
66 % Dh-SFM	4022.12±55.90 c	1.86±0.04 a	0.00±0.00	284.09±12.59 b	289.53±12.72 b
100 % Dh-SFM	3623.57±45.00 d	1.89±0.02 a	1.11±1.11	243.42±6.00 c	245.87±16.17 c
Probiotic					
W.O Probiotic	3967.55±91.58 b	1.71±0.05	0.55±0.55	341.35±27.75 b	345.05±27.66 b
With Probiotic	4128.19±88.19 a	1.68±0.05	0.55±0.55	366.32±28.35 a	370.91±29.10 a
Dh-SFM × Probiotic					
Control (-) W.O Probiotic	4205.77±27.35 b	1.53±0.01 b	2.22±2.22	424.51±16.79 a	422.08±25.07 b
Control (+) With Probiotic	4485.04±27.57 a	1.55±0.02 b	0.00±0.00	451.16±13.90 a	457.63±14.02 ab
33 % Dh-SFM W.O Probiotic	4160.78±31.04 b	1.51±0.01 b	0.00±0.00	436.34±8.98 a	442.94±18.03 ab
33 % Dh-SFM With Probiotic	4240.00±26.26 b	1.48±0.005 b	0.00±0.00	463.66±1.91 a	470.40±11.92 a
66 % Dh-SFM W.O Probiotic	3941.33±56.96 c	1.88±0.07 a	0.00±0.00	271.06±16.90 bc	276.42±17.01 cd
66 % Dh-SFM With Probiotic	4102.92±76.52 b	1.83±0.07 a	0.00±0.00	297.13±18.36 b	302.63±18.58 c
100 % Dh-SFM W.O Probiotic	3562.34±73.99 d	1.92±0.02 a	0.00±0.00	233.49±6.60 c	238.74±6.64 d
100 % Dh-SFM With Probiotic	3684.81±47.71 d	1.86±0.04 a	2.22±2.22	253.34±6.17 c	253.00±9.78 d

Different letters in the column represent significant differences at the probability level ($p \leq 0.05$). **Dh-SFM** is de-hulled sunflower meal; **W.O** is without; **EPEF** is European Production Efficiency Factors.

Dressing Percentage and internal viscera:

Table (4) shows that there were no significant differences between the control treatment and the second and third treatment in the dressing percentage, while a significant decrease ($p \leq 0.05$) was observed in the dressing percentage upon total replacement in the fourth treatment (100% Dh-SFM). The results also indicate that there are no significant differences in the relative weight of the liver and heart between the treatments under the influence of the factors under study. An increase in the relative weight of the gizzard and a decrease in the relative weight of belly fat were observed significantly ($p \leq 0.05$) in the replacement treatments (66 and 100% Dh-SFM) compared with the control treatment. As for the effect of the interaction

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between the replacement of de-hulled sunflower meal with the addition of soybean meal and the addition of the probiotic, it is noted that the refining percentage was not affected by the addition of the probiotic at the levels of partial and total replacement of the de-hulled sunflower meal compared to the corresponding levels free of the probiotic. Perhaps the reason for the significant decrease in the dressing percentage in the total replacement treatment of sunflower meal (100%) is due to the low live body weight of these treatments because there is a direct relationship between the live body weight and the percentage of dressings, birds with high body weight have higher percentages of recovery than birds with low body weight [24]. Likewise, the reason for the increase in the relative weight of abdominal fat in the treatments containing the probiotic may be attributed to the ability of the bacteria included in its composition to exclude pathogenic bacteria and to increase the number of lactobacilli that are found in the intestinal flora naturally [5], which work to produce volatile fatty acids (VFA) and short-chain fatty acids (SCFA), which can be used as an energy source by the host (the bird), which leads to an increase in the amount of excess energy that the body needs, which is converted into fat to eventually accumulate in the abdominal adipose tissue.

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Table (4). Effect of substituting de-hulled sunflower meal as a substitute for soybean meal with or without the Probiotic in dressing percentage and internal viscera of broiler chickens.

Treatments	Dressing percentage (%)	Relative weight (%)			
		Liver	Heart	Gizzard	Abdominal Fat
Level of Dh-SFM					
Control	73.93±0.36 a	2.31±0.03	0.51±0.07	c1.67±0.04	0.75±0.07 a
33 % Dh-SFM	73.06±0.81 a	2.48±0.03	0.47±0.01	1.72±0.03 c	0.75±0.01 a
66 % Dh-SFM	72.61±0.32 a	2.36±0.03	0.57±0.05	1.99±0.06 b	0.65±0.02 b
100 % Dh-SFM	68.95±0.17 b	2.65±0.08	0.48±0.01	2.19±0.06 a	0.49±0.02 c
Probiotic					
W.O Probiotic	72.47±0.60	2.40±0.03	0.50±0.01	0.08±1.92	0.59±0.03 b
With Probiotic	71.80±0.69	2.50±0.06	0.51±0.01	1.87±0.04	0.73±0.04 a
Dh-SFM × Probiotic					
Control (-) W.O Probiotic	74.73±0.02 a	2.33±0.01	0.51±0.01	1.60±0.01 c	0.59±0.06 c
Control (+) With Probiotic	73.12±0.01 a	2.30±0.06	0.51±0.01	1.75±0.08 c	0.92±0.01 a
33 % Dh-SFM W.O Probiotic	73.17±0.24 a	2.40±0.01	0.44±0.01	1.65±0.01 c	0.73±0.01 b
33 % Dh-SFM With Probiotic	72.95±1.80 a	2.56±0.04	0.50±0.01	1.79±0.02 c	0.77±0.03 b
66 % Dh-SFM W.O Probiotic	72.67±0.37 a	2.27±0.01	0.56±0.08	2.11±0.07 ab	0.59±0.01 c
66 % Dh-SFM With Probiotic	72.56±0.63 a	2.45±0.02	0.57±0.01	1.87±0.08 bc	0.71±0.05 b
100 % Dh-SFM W.O Probiotic	69.31±0.12 b	2.59±0.02	0.50±0.06	2.31±0.02 a	0.44±0.02 d
100 % Dh-SFM With Probiotic	68.59±0.04 b	2.71±0.18	0.46±0.01	2.07±0.06 ab	0.53±0.08 cd

Different letters in the column represent significant differences at the probability level (p≤0.05). **Dh-SFM** is de-hulled sunflower meal; **W.O** is without.

Intestine microbial content (gut biology):

It is noted from the results of the statistical analysis (Table 5) that there was a significant increase (P≤0.05) in the number of pathogenic bacteria (*Escherichia coli*) linearly with an increase in the levels of substitution of de-hulled sunflower meal in the third treatment (66%) and the fourth (100%) compared with the control treatment. It is also noted that there was a significant decrease (p ≤ 0.05) in the number of beneficial bacteria (lactobacilli) in all replacement treatments (33, 66, and 100%) compared with the control treatment. As for the effect of the interaction between the replacement of de-hulled sunflower meal instead of soybean meal and the addition of the probiotic, A significant decrease (P≤0.05) in the number of *Escherichia coli* bacteria and a significant increase (P≤0.05) in the number of Lactobacilli bacteria were observed in the case of overlap between the substitution levels of de-hulled sunflower meal partially (66%) and

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completely (100%) with the addition of the probiotic compared with the same level corresponding without addition. The reason for the significant increase in the number of pathogenic bacteria (*Escherichia coli*) and the significant decrease in the number of beneficial bacteria (lactobacilli) in all substitution treatments of sunflower meal can be attributed to an increase in the level of non-starchy polysaccharides and their negative effect on the digestion and absorption of nutrients in the small intestine. This impediment in the process of digestion and absorption leads to an increase in fermentation in colonies of harmful bacteria in the gastrointestinal tract [17], which negatively affects the productive performance of the bird. as pathological bacteria compete with beneficial bacteria and the host for nutrients and produce substances such as ammonia that can affect negatively on the overall health of the bird [10]. On the other hand, the reason for the increase in the numbers of beneficial bacteria (lactobacilli) may be due to the ability of the probiotic to change the type and number of gut microflora, by reducing the negative effects of non-starchy polysaccharides and inhibiting harmful bacteria. [14] confirmed that the specificity of the action of the probiotic in bringing about a state of microbial balance is due to several mechanisms, including the role of the probiotic in the competition of pathogenic microorganisms for nutrients and thus inhibiting their growth and reproduction, and the competition of pathogenic bacteria on the receptor sites located on the epithelial cells lining the gastrointestinal tract and sticking to them, thus facilitating the excretion of pathogenic bacteria with waste out of the body.

Intestinal tissue anatomical structure (intestinal morphology):

The results in Table (5) indicate that there are no significant differences in the villus length and the ratio of villus length to the crypt depth between the control treatment and the second treatment (33% Dh-SFM), and with an increase in the replacement rate in the third and fourth treatments (66 and 100% Dh-SFM). A significant decrease ($P \leq 0.05$) was observed in all of these traits and a significant increase ($P \leq 0.05$) in the crypt depth compared with the control treatment. As for the effect of the interaction between the replacement of de-hulled sunflower meal instead of soybean meal and the addition of the probiotic, The results of the statistical analysis showed a significant improvement ($P \leq 0.05$) in the villus length and the ratio of villus length to the crypt depth, as a result of adding the probiotic to the replacement coefficients of de-hulled sunflower meal at levels (33, 66, and 100%) compared to the corresponding levels without addition. The positive effect of adding the probiotic on the intestinal morphology (intestinal morphology) of the jejunum and maintaining the stability of the internal environment of the bird may be due to the bacteria involved in the formation of the probiotic's secretion of enzymes that digest non-starchy polysaccharides and reduce its negative impact on the morphology of the intestine. The mechanism lies in the action of the probiotic on the production of oligosaccharides (XOS), which play an important role in increasing the number of beneficial bacteria, including lactobacilli, as these bacteria produce amino acids, vitamins, mineral elements, and short-chain fatty acids, which are an important source for the proliferation of epithelial cells. and its perpetuation and renewal continuously, as well as its role in reducing the production of ammonia in the intestinal lumen [29], which leads to an increase in the height of the villi, and the perpetuation of lepercohn cells and goblet cells that secrete mucus.

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Table (5). Table 4. Effect of substituting de-hulled sunflower meal as a substitute for soybean meal with or without probiotics on the microbial content and morphology of intestine of broiler chickens.

Treatments	* *Escherichia Coli*	* *Lactobacillus*	Villus Length (µm)	Crypts Depth (µm)	VL:CD (µm)
Level of Dh-SFM					
Control	5.12±1.12 c	730.50±122.15 a	1179.74±29.28 a	155.60±4.52 b	7.58±0.28 a
33 % Dh-SFM	12.37±3.09 bc	610.00±98.89 b	1152.48±32.20 a	159.01±4.55 b	7.24±0.30 a
66 % Dh-SFM	17.37±7.08 b	446.50±130.51 c	992.59±46.64 b	216.32±6.22 a	4.58±0.29 b
100 % Dh-SFM	43.87±18.47 a	272.00±101.96 d	881.57±39.74 c	221.67±8.71 a	3.97±0.29 c
Probiotic					
W.O Probiotic	32.06±10.06 a	318.75±63.19 b	970.89±39.45 b	198.69±9.04 a	4.88±0.40 b
With Probiotic	7.31±1.48 b	710.75±68.00 a	1132.29±25.46 a	177.61±6.64 b	6.37±0.36 a
Dh-SFM × Probiotic					
Control (-) W.O Probiotic	6.50±1.50 c	519.00±8.00 d	1132.86±46.34 b	161.68±6.30 d	7.00±0.30 b
Control (+) With Probiotic	3.75±1.25 c	942.00±3.00 a	1226.62±24.77 ab	149.52±5.82 d	8.20±0.29 a
33 % Dh-SFM W.O Probiotic	17.25±2.75 bc	439.00±10.00 e	1113.82±32.53 b	166.20±7.01 d	6.70±0.23 b
33 % Dh-SFM With Probiotic	7.50±1.50 c	781.00±10.00 b	1191.14±53.49 ab	151.82±4.29 d	7.84±0.45 a
66 % Dh-SFM W.O Probiotic	29.50±2.50 b	221.00±10.00 f	861.66±30.52 d	226.84±8.89 ab	3.79±0.21 d
66 % Dh-SFM With Probiotic	5.25±0.75 c	672.00±20.00 c	1123.52±16.98 ab	205.80±6.31 bc	5.45±0.10 c
100 % Dh-SFM W.O Probiotic	75.00±10.00 a	96.00±2.00 g	775.24±34.89 d	240.04±12.3 a	3.23±0.18 d
100 % Dh-SFM With Probiotic	12.75±3.25 c	448.00±5.00 e	987.90±15.38 c	203.30±4.51 c	4.85±0.17 c

Different letters in the column represent significant differences at the probability level ($p \leq 0.05$).
***** Bacterial count (cells x 10^4/g of intestinal content). **Dh-SFM** is de-hulled sunflower meal; W.O is without. **VL:CD** is Villus Length to Crypts Depth. **µm** is Micrometer.

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