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CLINICOPATHOLOGICAL EFFECT OF PROBIOTICS ON ENTERIC DISEASES IN BROILER CHICKS

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A B S T R A C T

80 Cobb chicks one day old were used in this study. The chicks were divided into four groups (n=20/ group). Group (A) and (B) were experimentally infected with *Campylobacter jejuni* (*C. jejuni*) at the 4th day of age, while group (C) and (D) were experimentally infected with *Clostridium perfringens* type (A) at the 10th day of age. Group (A) and (C) fed on balanced ration, while group (B) and (D) were fed balanced ration supplied with Enterococcuse faecium (E.faecium) and Saccharomyces cervisiae (S. cervisiae) probiotics. Blood samples were collected at 2nd, 4th and 6th weeks of age to examine haematological, biochemical and immunological parameters as well as the changes in acute phase parameters. The results of this study showed that there were significant increase in TLC, heterophilis and lymphocytes number, albumin, total proteins, calcium and HI antibody titer in group (B) and (D) compared to group (A) and (C) respectively, on the other hand there were significant decrease in ESR, fibrinogen concentration, cholesterol, glucose, creatinine, uric acid and AST activity. It is concluded that the use of E. faecium and *S. cervisiae* probiotics improve haematological, biochemical, immunological parameters and acute phase parameters in broiler chicks infected with *C. jejuni* and *Clostridium perfringens*.

KEY WORDS: Campylobacter jejuni, Clostridium perfringens, Enterococcuse faecium, Saccharomyces cervisiae

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1. I N T R O D U C T I O N

In the recent decades, deficiencies in feed formulation and management practices have been masked by the routine use of antibiotic growth promoters (AGP). However, the ban of AGP has driven the implementation of alternative strategies in order to maintain health and performance status and optimizing digestion in poultry and to destroy the pathogenic dangerous enteric diseases, which caused illness to human as *Campylobacter* and *Clostridium*. In order to prevent this infectious diseases, live microbial cultures, named probiotics, can be administrated to chicks to optimize the colonization and composition of gut micro-flora and have a stimulatory effect on digestive process and the immunity [10], improving feed intake and digestion, neutralizing enterotoxins, altering metabolism by increasing digestive enzyme activity [18] and reduce the presence of *Campylobacter jejuni* (*C. jejuni*) [34] and suppressing *Clostridial* infection [29]. Therefore the aim of this study was to examine the effect of feeding Enterococcuse faecium (*E. faecium*) and Saccharomyces cervisiae (*S. cervisiae*) probiotics on improving the changes induced by experimental infection of *C.*

jejuni and *Clostridium perfringens* (*C. perfringens*) on haematological, biochemical, immunological parameters and acute phase parameters in broiler chicks.

2. MATERIAL AND METHODS

2.1. Birds:

A total of 80 one day old chicks (Cobb breed) were used. They were obtained from (Alahram Company).the chicks were randomly allocated into four groups (20 chicks / group). The chicks were housed in clean well-ventilated previously fumigate room. The room floor was bedded by fresh clean chopped wheat straw forming a deep litter of 3.5 cm depth, which was turned over weekly and changed every two weeks. Each group of bird was provided by suitable feeder and water. The broiler chicks were fed on well-balanced diet prepared from a corn-soybean meal based diet. Starter diet was given till the 20^{th} day of age after that chicks were fed on finisher diet which was given from the 21^{st} day till the end of the experiment. The chicks were vaccinated against most common viral diseases, which infect the broiler chicks.

2.2. Probiotics

Avi-lution® was applied in a dose of 30 gm /1000 liter in drinking water of group (B) and (D) from 1^{st} day of age till the end of experimental period. Avi-lution® contains *E. faecium* 1.5×10^{10} CFU/g and *S. cervisiae* 1×10^{9} CFU/g.

2.3. Experimental infection microorganism

2.3.1. *Campylobacter jejuni:*

At the 4^{th} day of age, each chick of group (A) and (B) was orally infected with 0.1 ml saline containing (2.5×10^{8} CFU) *C. jejuni*.

2.3.2. Clostridium perfringens:

At the 10^{th} day of age, each chick of group (C) and (D) was infected via subcutaneous route with 0.1 ml saline containing (9×10^{8} CFU) *C. perfringens* type (A).

2.3. Blood sampling

Two blood samples were collected from heart. One of them placed on tube contain EDTA as anticoagulant for hematological examination and the other put in plane tube for separation of serum to be used in biochemical and immunological tests .The blood were collected at 2^{nd}, 4^{th} and 6^{th} weeks of age.

2.4. Clinicopathological assays

2.4.1. Hematological assay:

It was performed according to [9] and it included TLC, DLC and ESR.

2.4.2. Biochemical assays:

Total proteins, albumin, serum electrophoresis, total cholesterol, AST, glucose, creatinine, uric acid, calcium, fibrinogen were assayed by using commercial kits (Stanbio-laboratory, USA).

2.4.3. Immunological test:

Haemagglutination inhibition test was performed on serum samples according to [20].

2.5. Statistical analysis:

The obtained data were analyzed with one way ANOVA test using statistical software package SPSS for Windows (version 11.).

3. RESULTS

Leukogram showed significant increase in TLC in group (B) and (D) at 6th week of age compared to group (A) and (C) respectively. Lymphocytes and heterophilis number showed significant increase at 4th week of age till the end of experimental period as showed in the table. 1. Regarding to acute phase parameters, significant decrease in fibrinogen and ESR were recorded in group (B) and (D) at 6th week of age compared to the results of group (A) and (C) respectively, while albumin results showed significant increase as showed in the table 2. In group (B) and (D) significant increase was recorded in total proteins concentration at 6th week of age compared to group (A) and (C) and significant increase in Alpha one globulin concentration at 6th week of age in group (B), while group (D) showed significant increase at 4th week of age.

Table 1 Leukogram of different groups

Age (Week)	Group (A)	Group (B)	Group (C)	Group (D)
	---------- Total leukocytes count ($\times10^3$/µl) ----------			
2nd week	22.00±4.30 [a]	23.00±4.30[a]	21.80±3.40 [a]	23.40±4.56 [a]
4th week	23.00±3.87 [a]	23.80±1.30 [a]	26.60±1.67 [a]	27.80±2.38[a]
6th week	24.00±1.50 [a]	30.00±1.60[b]	27.00±2.90 [a]	32.00±2.30[b]
	---------- Lymphocytic count ($\times10^3$/µl) ----------			
2nd week	11.20±0.83[a]	12.00±1.41[a]	11.6±1.14 [a]	11.60±1.14[a]
4th week	11.60±1.81[a]	12.00±1.64[b]	13.00±0.70 [a]	14.00±3.53[b]
6th week	13.00±1.00 [a]	18.00±0.70[b]	14.20±2.16 [a]	19.00±1.00[b]
	---------- Heterophilic count ($\times10^3$/µl) ----------			
2nd week	8.40±1.14 [a]	9.00±0.83[a]	7.20±1.78 [a]	8.20±1.92[a]
4th week	8.40±1.90 [a]	11.00±1.10 [b]	11.00±1.50 [a]	12.00±1.50 [b]
6th week	10.40±1.67 [a]	12.00±1.87[b]	10.20±1.10 [a]	12.20±1.48[b]
	---------- Monocytic count ($\times10^3$/µl) ----------			
2nd week	1.800±0.283[a]	1.800±0.149[a]	1.600±0.140 [a]	1.400±0.116[a]
4th week	1.800±0.288 [a]	1.600±0.140[a]	1.600±0.140 [a]	1.600±0.140[a]
6th week	1.600±0.216 [a]	1.400±0.241[a]	1.200±0.303 [a]	1.200±0.303[a]

Means (± SD) with different superscripts (a and b) within a row are significantly different at $P < 0.05$.

Table 2 Changes in acute phase parameters in different group

Age /week	Group (A)	Group (B)	Group (C)	Group (D)
	---------- Fibrinogen (g/dl) ----------			
2nd week	0.72±0.14[a]	0.65±0.13[a]	0.73±0.69[a]	0.46±0.07[a]
4th week	0.76±0.12[a]	0.68±0.15[a]	0.78±0.10[a]	0.66±0.04[a]
6th week	0.70±0.17[a]	0.56±0.09[b]	0.78±0.69[a]	0.57±0.07[b]
	---------- Albumin (g/dl) ----------			
2nd week	0.88±0.08[a]	0.92±0.03[a]	0.84±0.04[a]	0.87±0.03[a]
4th week	0.87±0.02[a]	0.89±0.00[a]	0.84±0.04[a]	0.89±0.03[a]
6th week	0.80±0.02[a]	0.89±0.02[b]	0.78±0.02[a]	0.88±0.026[b]
	---------- ESR (mm) (1st hour) ----------			
2nd week	0.85±0.05[a]	0.85±0.06[a]	0.84±0.04[a]	0.84±0.04[a]
4th week	1.02±0.19[a]	1.00±0.12[a]	1.12±0.18[a]	1.00±0.22[a]
6th week	1.21±0.22[a]	0.98±0.14[b]	1.30±0.15[a]	1.08±0.15[b]

Means (± SD) with different superscripts (a and b) within a row are significantly different at $P < 0.05$.

However, Alpha 2 globulin concentration and beta globulins showed no significant changes in group (B) and (D) throughout of experimental period. Concerning to gamma globulins concentration, significant increase was reordered and significant decrease in A/G ratio at 6th week of age was observed in table.3.

On the other hand cholesterol level was significantly decreased at 4th week of age till the end of experiment and there were significant decreases in AST level in group (B) and (D) at 6th week of age (table 4). The results of serum glucose and creatinine showed significant decrease in group (B) and (D) at 6th week of age.

Table 3 Changes in serum total protein and serum electrophoretic pattern of different groups

Age (week)	Group (A)	Group (B)	Group (C)	Group (D)
	---------- Total proteins (g/dl) ----------			
2nd week	1.87±0.06[a]	1.99±0.35[a]	1.88±0.23[a]	1.97±0.29[a]
4th week	1.85±0.32[a]	1.93±0.14[a]	1.63±0.49[a]	1.96±0.35[a]
6th week	1.64±0.23[a]	2.31±0.12[b]	1.55±0.32[a]	2.05±0.42[b]
	---------- Alpha (1) globulins (g/dl) ----------			
2nd week	0.027±0.01[a]	0.061±0.03[a]	0.081±0.02[a]	0.080±0.01[a]
4th week	0.145±0.05[a]	0.175±0.04[a]	0.111±0.01[a]	0.189±0.01[b]
6th week	0.016±0.00[a]	0.143±0.09[b]	0.038±0.03[a]	0.040±0.01[a]
	---------- Alpha (2) globulins (g/dl) ----------			
2nd week	0.355±0.04[a]	0.450±0.13[a]	0.378±0.04[a]	0.398±0.03[a]
4th week	0.343±0.04[a]	0.346±0.03[a]	0.255±0.052[a]	0.307±0.06[a]
6th week	0.389±0.04[a]	0.416±0.06[a]	0.319±0.02[a]	0.255±0.03[a]
	---------- Beta globulins (g/dl) ----------			
2nd week	0.088±0.17[a]	0.128±0.03[a]	0.134±0.05[a]	0.153±0.09[a]
4th week	0.133±0.05[a]	0.190±0.44[a]	0.163±0.02[a]	0.167±0.02[a]
6th week	0.089±0.00[a]	0.100±0.02[a]	0.062±0.01[a]	0.132±0.01[a]
	---------- Gamma globulins (g/dl) ----------			
2nd week	0.344±0.03[a]	0.359±0.03[a]	0.376±0.02[a]	0.375±0.09[a]
4th week	0.338±0.02[a]	0.341±0.02[a]	0.316±0.04[a]	0.369±0.01[a]
6th week	0.313±0.03[a]	0.586±0.03[b]	0.371±0.61[a]	0.701±0.09[b]
	---------- A/G ratio ----------			
2nd week	1.08±0.18[a]	0.93±0.09[a]	0.86±0.08[a]	0.87±0.10[a]
4th week	0.90±0.05[a]	0.85±0.04[a]	0.99±0.08[a]	0.86±0.02[a]
6th week	0.99±0.09[a]	0.72±0.02[b]	0.99±0.10[a]	0.77±0.04[b]

Means (± SD) with different superscripts (a and b) within a row are significantly different at $P < 0.05$.

Table 4 Total cholesterol concentration and activity of serum Asparatate aminotransferase (AST) of different groups (means ± SD).

Age (Week)	Group (A)	Group (B)	Group (C)	Group (D)
	---------- Total cholesterol (mg/dl) ----------			
2nd week	127.21±17.57[a]	113.76±14.36[a]	126.91±11.55[a]	117.95±16.18[a]
4th week	130.92±11.12[a]	113.45±12.45[b]	134.79±6.80[a]	115.34±13.32[b]
6th week	131.30±7.84[a]	113.12±9.75[b]	132.52±7.03[a]	114.19±20.49[b]
	---------- AST (U/L) ----------			
2nd week	192.40±8.64[a]	181.90±8.72[a]	199.20±10.32[a]	189.10±12.10[a]
4th week	193.20±13.16[a]	181.20±19.32[a]	201.20±8.46[a]	192.20±9.52[a]
6th week	194.60±14.04[a]	178.20±12.25[b]	202.20±8.25[a]	185.40±10.57[b]

Means (± SD) with different superscripts (a and b) within a row are significantly different at $P < 0.05$.

Table 5 Changes in serum glucose, creatinine, uric acid and calcium concentration of different groups

Age (week)	Group (A)	Group (B)	Group (C)	Group (D)
	---------- Glucose (mg/dl) ----------			
2nd week	340.00±14.40[a]	335.29±19.72[a]	357.47±18.45[a]	340.67±11.12[a]
4th week	381.51±12.16[a]	353.44±21.76[a]	382.35±26.90[a]	367.89±17.22[a]
6th week	366.89±30.52[a]	323.50±28.23[b]	387.56±21.46[a]	349.91±18.51[b]
	---------- Creatinine (mg/dl) ----------			
2nd week	1.40±0.15[a]	1.28±0.22[a]	1.40±0.26[a]	1.28±0.27[a]
4th week	1.44±0.15[a]	1. 30±0.20[a]	1.60±0.10[a]	1.50±0.10[a]
6th week	1.52±0.08[a]	1.20±0.18[b]	1.62±0.08[a]	1.20±0.20[b]
	---------- Uric acid (mg/dl) ----------			
2nd week	17.17±2.03[a]	14.97±1.14[a]	18.22±0.93[a]	15.88±1.04[a]
4th week	19.99±1.75[a]	17.31±3.00[b]	19.32±0.73[a]	17.82±0.95[b]
6th week	19.89±2.41[a]	16.71±1.80[b]	19.07±1.15[a]	15.81±1.11[b]
	---------- Calcium (mg/dl) ----------			
2nd week	5.64±0.74[a]	6.03±0.37[a]	5.24±0.46[a]	5.87±0.62[a]
4th week	5.40±0.65[a]	6.10±0.36[a]	5.00±0.70[a]	6.49±0.72[a]
6th week	5.24±0.28[a]	6.00±0.63[b]	5.10±0.88[a]	6.61±0.45[b]

Means (± SD) with different superscripts (a and b) within a row are significantly different at $P < 0.05$

Table 6 Results of Haemagglutination inhibition (HI) test of experimentally infected chicks and control group (means ± SD)

Age (week)	Group (A)	Group (B)	Group (C)	Group (D)
	---------- HI antibody titer ----------			
2nd week	3.4±1.26	4.0±1.22	3.5±1.00	3.7±1.09
4th week	3.4±1.75	3.5±0.00	3.8±1.62	3.8±1.66
6th week	3.6±1.01	3.7±0.142	3.6±1.94	3.9±1.84

However the concentration of uric acid showed significant decrease at 4th and 6th weeks and significant hypercalcaemia was observed at 6th week of age in table 5. Concerning to antibody titer against Newcastle virus, group (B) and (D) showed HI antibody titers higher than those of group (A) and (C), respectively at 2nd week of age till the end of experimental period as showed in table 6).

4. DISCUSSION

Recently, researchers have found that the use probiotics shown efficacy against food borne pathogens such as *Salmonella spp.*, *C. jejuni, C. botulinum, C. perfringens*, pathogenic strains of *Escherichia coli* and *Yersinia enterocolitica* [23] and explained that to the inhibitive action of probiotics against pathogens which mediated by competition for receptors on the gut mucosa, competition for nutrients, production of antibacterial substances and stimulation of immunity [28].

In the present study significant leukocytosis and lymphocytosis were reported in group (B) and (D) at 6th week of age and significant heterophilia at 4th week of age till the end of experimental period. Our data agree with those reported by [1, 11] who observed lymphocytic leukocytosis in bacterial infected broiler chicks and probiotics administrated. Our results of leukogram are disagreed with that obtained by [26] who mentioned that the use of probiotics had no significant effect on total leukocytic count. Leukocytosis could be attributed to the effect of probiotics in stimulation of bone

marrow to produce more leukocytes [21]. Also probiotics stimulate immunity to infection by boosting interferon production immunoglobulin concentration and macrophage activity. On the other hand, lymphocytes are the bulky leukocyte in the peripheral blood of most normal chicken that play a major role in the humeral and cell mediated immunity of bird, therefore, lymphocytosis is suggestive of immunogenic stimulation [33].

Acute phase proteins are group of plasma proteins which are synthesized in the liver and released into blood stream by a variety of stimuli including inflammation and bacterial infection [17]. Acute phase response may be increased (positive acute phase proteins) or deceased (negative acute phase proteins) during inflammatory disorders [25]. Gruys et al. [13] concluded that in veterinary medicine, determination of acute phase proteins gives valuable clinical information on infection and inflammatory condition.

Our results showed that group (B) and (D) showed significant decrease in fibrinogen and ESR results at 6^{th} week of age till the end of experimental period. This decrease may be as a result of decease in the degree of inflammation and infection which was achieved by using of *E. faecium* and *S. cervisiae*. Referring to albumin; the concentration of albumin in group (B) and (D) revealed significant increase at 6^{th} week of age. The increase in concentration of albumin may be related to the improvement of absorption of intestine and liver production of albumin as a response of improvement of intestine and liver lesions. Our result agrees with earlier reports [8] observed that no significant increases in acute phase response when administrated probiotics in drinking water. Our result indicated that infected groups with *Campylobacter* and *Clostridia* showed acute phase response. These results are in fit with the concept of [24] who reported that whole bacterial cells are shown to induce production of pro-inflammatory cytokines, such as tumor necrosis factor α and interleukin 6 which stimulated the acute phase response [4] In contrast, probiotics bacteria mediate suppression of lymphocyte proliferation and cytokine production by T cells [30, 31] and therefore acute phase response is less severe.

Concerning to total proteins, group (B) and (D) showed significant increase in total proteins at 6^{th} week of age. Our data disagree with those obtained previously [6] which reported that the probiotics cause no significant changes in total proteins. Hyperprotenima may due to the role of probiotics in improving the sings of inappertance and improvement the lesion in liver and intestine therefore improve absorption and metabolism of protein. Regarding to electrophoric patterns of group (B) and (D), alpha 1 showed significant increase at 6^{th} week of age in group (B), but group (D) showed significant increase at 4^{th} week of age, while alpha 2 and beta globulins showed no significant changes throughout the experimental period. Improvement of liver lesion in group (B) and (D) by the action of probiotics may be the main cause of increase of alpha 1 globulins. The forementioned results agree with the results achieved by previous authors [1, 11] who achieved increase in alpha globulins in probiotics administrated broiler chicks and partially agree with earlier studies [6] mentioned that probiotics cause no significant changes in globulins. The data of group (B) and (D) showed significant increase in gamma globulins at 6^{th} week of age. Increase of gamma globulins in these groups may be related to the stimulatory effect of probiotics in increase of humeral immunity [3].

Our result showed significant decrease in total cholesterol results from the 4^{th} week [14] of age till the end of experiment in group (B) and (D), respectively. These results agree with [1]. On the same trend it has been recently reported that the reduction of serum cholesterol were

observed by supplementation of probiotics in the diet of broilers. This decrease may be due to the ability of the probiotics bacteria *E. faecium* and *S. cervisiae* to assimilate the cholesterol present in the GIT for their own cellular metabolism thus reducing the amount absorbed [22]. Moreover, the uptake of cholesterol by desirable bacterial cells and co-precipitation with de-conjugated bile salts caused low cholesterol level [12]. The previous mentioned results are disagree with previous study [2] who found that no significant changes in cholesterol in broiler, which received probiotics.

There was significant decrease in AST activity at 6^{th} week of age in group (B) and (D). These data indicates improvement of liver lesion in groups, which administrated probiotics. Our findings disagree with [1, 11, 15] who achieved no significant changes in AST in probiotics treated chicks. Regarding to concentration of serum glucose, the results in group (B) and (D) showed significant hypoglycemia at 6^{th} week. This decrease could be attributed to the effect of probiotics in reducing the bacterial infection and stress and tissue damage. The above results disagree with those reported formerly [5, 11] found marked increased in glucose concentration in probiotics treated group and that there is no significant changes in glucose concentration in probiotics treated group. Concerning to the calcium concentration, significant hypercalcaemia was reported in group (B) and (D) at 6^{th} week of age. These data are agreed with these reported in previous studies [1, 27] found a marked increase in calcium concentration in probiotics treated group.

Regarding to uric acid and creatinine concentration, the data of group (B) and (D) showed significant decrease in uric acid at 4^{th} week of age till the end of experiment in group (B) and at 6^{th} week of age in group (D). This reduction in uric acid and creatinine concentration may be due to the effect of probiotics *E. faecium* and *S. cervisiae* in improving tissue damage and nutritional status of broiler chicks. Our results agree with earlier reports [1, 11, 15, 16] that mentioned that no significant changes in uric acid and creatinine in probiotics administrated chicks.

With respect to the antibody titers against Newcastle virus, group (B) and (D) showed HI antibody titers higher than those of group (A) and (C), respectively at 2^{nd} week of age till the end of experimental period. This may be attributed to the effect of probiotics in stimulation of immune system and production of immunoglobulin (A) [7]. Our data are agreed with [3] who reported that probiotics had stimulatory effect of humeral immunity. On the same trend [19] detected that antibody production against Newcastle disease virus (NDV) in a group of broiler chicks treated with probiotics was significantly higher 10 days post immunization than that in an untreated group. Our results disagree with some studies [1, 32] mentioned that the probiotics had no effect on humeral immunity.

5. CONCLUSION

It is concluded that the *E. faecium*, *S. cervisiae* probiotics improve heamatological, biochemical and immunological parameters and acute phase parameters in broiler chicks infected with *C. jejuni* and *C. perfringens*.

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تأثير البروبيوتك علي الامراض المعويه في بداري التسمين

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الملخص العربى

اجريت هذه الدراسه باستخدام 80 كتكوت من سلاله الكب عمر يوم واحد وتم تقسيم الكتاكيت الي اربع مجموعات (20 كتكوت / المجموعه) . وقد تم اجراء عدوي تجريبيه للمجموعه الاولي والثانيه بالكامبيلو بكتر جيجيناي عند عمر اربع ايام اما المجموعه الثالثه والرابعه فقد تم اجراء عدوي تجريبيه بالكولوستيريديا بريفرنجيس عند عمر 10ايام . وقد تم تغذيت المجموعه الاولي والثالثه علي عليقه متزنه اما المجموعه الثانيه والرابعه فقد تغذت علي عليقه متزنه مع اضافهالبروبيوتك المحتوي علي الانتيروكوكس والسكارواميسين سيرفسي الي مياه الشرب . وقد تم تجميع عينات من الدم في الاسبوع الثاني والرابع والسادس من عمر الكتاكيت لفحص التغيرات الدمويه والبيوكيميائيه والمناعيه . واظهرت نتائج هذه الدراسه ان هناك زياده معنويه في العدد الكلي لكرات الدم البيضاء والهتيروفيل والخلايا الليمفاويه والزلال والبروتينات الكليه والكالسيوم وتركيز الاجسام المضاده ضد تحصين النيوكسل في المجموعه الثانيه والرابعه مقارنة بالمجموعه الاولي والثالثه علي التوالي. من ناحيه اخري كان هناك انخفاض ملحوظ في سرعه ترسيب الدم وتركيز الفيبرينوجين والكلولستيرول والجلوكوز والكرياتينين وحمض البوليك ونشاط انزيم الاسبرتيتت امينو ترانس فريز. وخلصت الدراسه علي ان استخدام البروبيوتك المحتوي علي الانتيروكوكس والسكاروا ميسين سيرفيساي تعمل علي تحسين التغيرات الدمويه والبيوكيميائيه والمناعيه في بداري التسمين المصابه بعدوي الكمبيلوبكتر جيوجيناي والكلوستيراديم بيرفيرنجيس.

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