

# A PHARMACOLOGICAL COMPARISON BETWEEN the PROBIOTIC PREPARED by the LACTOBACILLUS ACIDOPHILUS BACTERIA and NITAZOXANID in ANIMALS INFECTED WITH CRYPTOSPORIDIOSIS

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## **Abstract:**

The study was conducted for the period from April 2016 to March 2017. The study of lactobacilli, which was prescribed by acidophillus Lactobacilli (Probiotics), was included in the treatment of the parasite *Cryptosporidium parvum* compared to nitazoxanid, based on several criteria including therapeutic efficacy and some blood criteria. The number of egg cyst in the first day of the treatment reached 6640 syst / eggs with a therapeutic efficiency of 22.9%. In the first and second concentrations, the ratio reached 7180, 6460 and 5560 with a therapeutic efficiency of 16.7, 25.0 and 35. The number of egg cyst continued to decline to the fifth day. The number of eggs cyst was 40 cysts / eggs and 99.4% The number of red blood cells in total infection compared to control was  $5.782 \times 10^9 / L$ , while there was no significant difference in control. The results showed no significant differences in hemoglobin ratios and the volume of red blood cells between total negative control, total probiotic treatment group, and groups treated with drugs in concentrations II and III, and in the number of white blood cells, granulocyte and monocyte were no differences Between the probiotic group and the negative control group, while all the remaining groups decreased.

**Key words:** *Cryptosporidium parvum*, bioenergy. Probiotic.

## **INTRODUCTION**

Cryptosporidiosis is the most common human pathogen (Checkley *et al*, 2015) This disease is the second most common cause of rotavirus in global causes of death among children under 2 years of age, causing mild to severe diarrhea (Shirley *et al*, 2012) and causing 5-10 million Case of death annually (Nemes, 2009). Although the spawning parasite was discovered in 1907 by the Tyzer, it was discovered only as a cause of human infection in 1976 and in 2004 it was added to the World Health Organization's "Neglected Diseases Initiative", which includes diseases that affect To people mainly in developing countries (Speich *et al*, 2016) (AL-Samarraie, *et al*, 2019) .

The parasite passes through a complex life cycle and the human or animal can put millions of eggs in the stool. Although egg cysts are unable to reproduce outside the host, they can survive in the environment for several months and resist disinfection, including chlorine. (Ehsan *et al*,2015) also The transmission and spread of infection is particularly associated with people who have frequent contact with animals and the use of manure and human stool as fertilizers (Ya Yang *et al*,2017). The egg cyst contains infective oocyst. Four sporozoites are introduced with feces to spread the infection and when eaten by the host, the egg sac releases 4 of the spores that bind to the superficial epithelial cells of the stomach, small intestine and colon. (Bowman *et al*,2003)

It has been scientifically proven that the continued and excessive use of antibiotics leads to the appearance of bacterial resistant. Therefore, alternative methods of antibiotics, which are used as therapeutic, prophylactic, or growth stimuli, have been directed to patients who want to use natural and safe methods of treatment (Maria *et al*,2015) . In recent years, there has been a wide trend towards the use of types of lactic acid bacteria isolated from human and animal sources as probiotics, and the emergence of a new generation of these biopharmaceuticals, either in known pharmaceutical forms or in the form of therapeutic food products in many countries in Europe, North America and the Far East. Its use in solving many problems related to food and consumer health. (Singhi & Kumar,2016) Hence the objectives of this study, which include:

Isolation and diagnosis of parasite *Cryptosporidium Parvum* of calves with diarrhea.  
Isolate and diagnose *Lactobacillus acidophilus* from milk and then prepare the lactic acid from this bacteria as a bio-booster (Probiotics).  
Study of the therapeutic effect of *L.acidophilus* on *C.parvam* parasite compared to nitazoxanid and some blood parameters.

## THE METHODS

Preparation of solutions, delays and dyes.

Carbol dye Fuxin alkaloid preparation according to (Baxby,1984), green malachite dye, saturated sucrose solution, according to (John & Petri ,2006) and PBS solution as describe by (Myers,1995), McFarland solution according to (Baron,1994) Central sugar fermentation as (Harrigan & McCance,1979) center of MRS-CaCO<sub>3</sub> (Henriksen & Pohlenz, 1981)

The parasite sample

*Cryptosporidium parvam* samples were collected from the dung of calves infected with diarrhea (Samarra village and AL-Alam city) on 2016/4/1. The parasite was diagnosed according to the method (Anderson , 1981), as well as by the flotation method of the diabetic solution. (Arrowood & sterling , 1987) the isolation of parasite and Eggs collaction don as (Savage,1984) The injection dose was determined by calculating the number of sacs and determining the injection dose by calculating the number of sacs in the amount of 0.5 ml and the dose was determined by 40000 sacs for each rabbit to be injected orally. The parasite bags were investigated in infected rabbit stools daily after the mixture to ensure the occurrence of parasitic infection. The preparation of several swabs of infected rabbit stools on a glass slide and examined under a microscope and watch cysts and calculated using the method of flotation with the diabetic solution.

#### Sample of bacteria

*Lactobacillus acidophilus* bacteria were isolated and implanted into the MRS medium. According to method of (Savage,1984). The morphological characteristics of the colonies were studied, the cultural and the biochemical tests of bacteria were carried out to ascertain the traits that would enable them to be used as a probiotics.

#### Cultural characteristics

The striking method was used to vaccinate bacterial models on selective media and incubated dishes at 37 ° C for 18-24 hours.

#### Catalytic examination

The isolates were grown in the center of the fluid MRS media and then transferred to 1 ml of sterile test tube and 1 ml of H<sub>2</sub>O<sub>2</sub> hydrogen peroxide was added (10% H / H) and the emergence of bubbles was observed.

#### Examination of ammonia composition of arginine

0.3% of Arginine-monohydrochloride was added to the MRS liquid medium and sterilized at 121 ° C for 20 minutes. Then healed the isolates and incubated for 7 days at 37 ° C. then took 1 mL of the fertilized medium and placed in a test tube and added 1 ml of the NSLER detector.

#### Carbohydrate fermentation test

Sugars fermentation test solated and developed after the replacement of the carbon source from the sugar fermentation medium in one of the sugars: Fructose, Glucose, Galactose Raffinose, Ribose, Lactose, Maltose and Saccharose by 1% The addition of sugars after the sterilization of the primary mean of the balance as the sterilization of sugars using sheets Millipor filtration size 0.22 mm and incubated isolates cultivated for 24-48 hours. Determination of positive fermentation through color change to yellow, after isolating the isolates, was indicated for identification during use in subsequent tests.

#### Testability of adhesion to *Lactobacillus* spores

This test was conducted according to (Fuller, 1975) as follows:

Part of the intestines were taken. In the current study, laboratory rabbits were used under sterile conditions. The intestines were opened and washed twice by 100 mL of (PBS) solution, pH 7.2. The intestinal lining was cut by a clean, sterile glass slide and suspended with 10 mL of phosphate solution .

Preparation of 10 ml of *Lactobacillu sacidophilus* bacteria on the liquid MRS medium. The germ cells were then mediated by the centrifuge device at 2500 cycles / min for 10 minutes. The liquid was separated from the germ cell deposit and completed with the same phosphate solution (PBS).

Take 0.4 ml of the epithelial cell suspension and add 0.1 mL of bacterial suspension and then put in a water bath for 30 min. At 35 ° C and at 20 ° C. After incubation, take drops and place on a glass slide and dye using a Gram and Gemsa stain. To determine adhesion, germ cells take violet while the epithelial cells appear red.

After the tests were carried out, the bacteria were counted by using the McFarland tube method as indicated in (Baron,1994). The bacteria isolated in the center (MRS) were frozen as indicated in (Contreras,1997) and then the lactic milk was prepared from the isolated

bacteria. (Jandal,2007) Lactate by means of a glucoma in the mouth directly by a special syringe prepared for this purpose by two milliliters twice a day.

#### Laboratory animals

In this study, adult male New Zealand white rabbits obtained from the Erbil Research Center were aged between 10-18 months and weighed between 1000-18,00 kg. The animals were placed in iron cages and the ground was sprayed with sawdust, Taking into account the daily cleanliness of cages and the exchange of sawdust, as well as sterilization of cages disinfectant every two days, as well as the animals fed ready-made in addition to providing water throughout the study.

#### Experience Design:

Forty-four laboratory animals were used for the treatment trials, randomly divided into 6 groups, 7 animals per group, the first group left untreated, the remaining five groups were injected with *cryptosporidium parvum* parasites  $4 \times 10^3$  cells / ml and monitored for 7 days until the state of diarrhea, and the division was as follows:

Control group (C-ve), This group was left untreated throughout the experiment.

Infection group (C + ve), Infection group This group was used to compare with the other groups in terms of infection development and was injected orally from the parasite *Cryptosporidium prvum*, 0.5 mL and  $4 \times 10^3$  cells / mL as a dose of experimental infection.

After the infection was confirmed, the infected rabbits were treated with nitazoxanid (3, 4 and 5 groups ) with 0.5, 1, 1.5 mg / 100 g respectively of animal weight. They were administered every 24 hours for five consecutive days.

Group 6: Animals were treated with a probiotic. The animal was given 2 ml orally from the dose prepared in paragraph (3-11). Each ml contains  $2 \times 10^5$  bacteria, every 12 hours for a week, taking into consideration the stool daily Calculate the number of sacs and calculate the therapeutic efficiency. And then monitor and analyze the stool for a period of 5 days to follow up the occurrence of the disease or not. The animals were monitored and monitored during the treatment period by examining or previewing the changes in the general state of the animal, the nature of the appetite, the nature of the stool, the temperature, and comparing it with the normal rates referred to by (Mohammed, 2000).and calculate the number of eggs cysts offered every 24 hours.

Number of eggs cysts (gm) number of eggs in tow roomsthe weight of the stool sample  $\times 200$

Since the weight of the model is 0.2 g, the number of egg bags  $\times 100$ , the method of (Ghazal ,1974). After the experiment was over, the animals were anesthetized by chloroform, and blood samples were withdrawn from the heart directly in a cardiac catheterization procedure.

## MATERIALS AND METHODS

The animals were monitored during the treatment period by examining or previewing the changes in the general state of the animal, the nature of the appetite the nature of the stool, the temperature, and comparing it with the normal rates referred to in (Mohammed, 2000). The rabbits' stools were examined daily in a manner that was used to determine whether or not the injury occurred.

The therapeutic efficiency was calculated according to (Xiao et al,1996) according to the following equation:

Therapeutic efficiency=

The average number of egg bags in control group -The average number of egg bagsthe  
everagr of the eggs cyst number in the control group

After the experiment was over, the animals were anesthetized by chloroform, and blood samples were withdrawn directly from the heart in a cardiac punch. Approximately 8-10 ml of blood was withdrawn and 1 ml of blood was placed in a container containing anticoagulant for blood testing (Complete blood film) by a self-blood analysis device.

## RESULTS AND DISCUSSION

### Diagnosis of parasite

The parasite was diagnosed with the Zell-Nelsen mutant, where the spherical egg sacs appeared red and shiny distinct from the green background of granular cytoplasm and contained 4-6 spores.

### Diagnosis of bacteria

*Lactobacilli* colonies are circular convex in green because of the presence of Bromo Cresol Green stain in the contents of the center axis and regular edges(Tietz, 2005). *Lactobacillus* is one of the most important microbiology used in Probiotic, effectively contributing to the ideal balance of microorganisms that form intestinal flora, which binds to the epithelial layer lining the digestive tract, and thus colonizes the gastrointestinal tract(Al-saadi,2010) *L.acidophillus* was a negative result of this test and is consistent with the study (Miles & Bootwala,2006). *Lactobacillus* is negative for this test due to its inability to produce Peroxidase, which analyzes the H<sub>2</sub>O<sub>2</sub> peroxide of H<sub>2</sub>O and O<sub>2</sub>, which appears in the form of bubbles giving a positive result.

The test of ammonia production of arginine was not given a positive result when *Lactobacillus* was exposed. On the analysis of arginine our results were identical to the results of(Nizamuddin & ahaa,2002). The carbohydrate test gave all the sugars used as a positive result except for the of the raffinoz and the ribose sugar gave a negative result. Isolation showed high susceptibility to adhesion through a large increase in the number of bacteria attached to epithelial cells. The compound responsible for the adhesion of *L. acidophilus* bacteria is the S S-layer protein interferon.

The wall of the germ cell, which is shared with the adhesion of the receptors located on the walls of the epithelial cell, which consists mainly of manganese sugar ) Nawaf, 2005)

The presence of this protein layer and its containment of glycoproteins, which consist of 0.9 - 1.4% carbohydrates and 3% water - resistant amino acids, play a key role in adhesion (Bernet,2009) ( Fadhil et al .,2019).

### Experimental Study

#### *Clinical signs*

The experimental infection in the animal groups (infection group) was shown to begin with the appearance of the clinical signs observed through changes in the animal's appetite, general condition and the nature of the stool. The results of the clinical signs were very clear when the infection group was given a dose of *carbotosporidium* parasite. Her movement did

not show a distinctive clinical marker in the precursors of the bio-booster as well as the animals in the control groups.

The use of biopharmaceuticals in feed processing increased the appetite of Prohibited animals. (Abas *et al*,2007) An increase in rabbit appetite was also observed in acid-resistant

*lactobacillus* bacteria.

The results of the examination the nature of the face's with the naked eye of the infection group in the first days showed that it was changed from the solid to the softer, and then when the infection developed, the stool appeared to be a liquid evidence of severe diarrhea, indicating the severity of the parasite. In one animal noted that her health had deteriorated, and die.

The

measuring of the temperature of experimental animals, a significant increase was observed since the third day of the experiment in the infection group at 39.4 ° C compared with the first day in which all groups with temperatures between 36 - 37.5 ° C, and continued rise in the animals of the infection group. And slow movement of animals in the infection group and this is a satisfactory sign (Muhammad,2008).

*Results of the study of the effect of nitazaxonide with different concentrations and the probiotic on the rate of deposition of cysts of parasite eggs in the rabbits.*

This study shows that the probiotic had the greatest effect on the therapeutic efficiency rate. The rate of cysts began to decrease gradually from the first day of treatment, reaching 6640 cysts of eggs and decreased in the second day to 1740 ,and continued to decrease to the fifth day of treatment, reaching 40 cysts of eggs with a therapeutic efficacy rate of 99.4% as shown in Table (1). The efficiency of the probiotic in treatment is attributed to the prevention of adhesion of parasite cyst to bacterial susceptibility to high density adhesion on the surfaces of epithelial cells. In addition, they produce a substance as a type of Biosurfacton called Sarlactin, which is a product of microorganisms that separates two bodies. To prevent the adhesion of most pathogens to host cells (Velra,1998). It was noted that the efficacy of *L. acidophilus* in the treatment of diarrhea was due to the prevention of adhesion of diarrhea-causing intestinal pathogens. (Vandenplas,1999) *L. reuteri* was used as a probiotic to control *C. parvum* infection because of its efficiency in rapidly clearing intestinal cells from the parasite out of the epithelial layer of the rat intestines infiction with parasite and it weakens the immune system (Alak,1997) Previous studies have also indicated that *Lactobacillus* species can bind to intestinal epithelial cells until they are planted on tissue farms and thus compete with the intestinal mucosa (Dorevitch *et al*,1015)

The efficiency of probiotic treatment follow the third concentration of nitazaxonid which decrease at the first day of treatment to 5550 as a efficiency treatment 35% and continuous decreasing to 2360,1080, 420, 60 cyst/eggs mg at the next day, third, fourth and, fifth day of treatment, respectively. The Treatment efficiency was 73.3%, 77.5%, 94.0% and 99.1%, respectively. In the same Table, the efficiency of the second concentration of nitazaxonide was found to reach 80.9%. On the fifth day, the number of egg cysts reached 80 cysts / eggs. This indicates the efficiency of the dose given to rabbits in reducing the number of parasite cysts raised with stool and this indicate full recovery. This is consistent with (Iqbal,2015). Nitazoxanide has been shown to be highly effective in the treatment of cryptosporidiosis patients as well as patients with immunosuppressive agents. Studies have shown a significant reduction in the incidence of egg sacs in nitazoxanide-treated mice for seven days and a 94% cure rate of 50-200 mg / kg bw(painter,2015). The same drug with a dose of 100-200 mg /

90% reduction in the incidence of cysts and recovery in small goats for five days (Valeur,2004), and recovery rate was 85% in calves infected with Cryptosporidiosis and treatment with nitazoxanide for four days. The interpretation of these contradictions is due to the composition of drugs by different manufacturers, Animal species used in research, as well as the difference in doses and the duration (schlagenhauf *et al*,2015)

Nitazoxanide's mechanism of action is to inhibit PFOR (pyruvate ferredoxin oxido reductase), the primary enzyme in the anaerobic metabolism of the parasite, and after the human takes the drug, its 2: 3 ratio is cast with feces (pensabene *et al*,2015).

Table (1) Effect of nitazoxanide with different concentrations and probiotic on the rate of egg cysts in rabbits infection with *Cryptosporidium* parasites

	Eggs cysts number after First day of treatment	efficiency	Eggs cysts number after First second day of treatment	efficiency	Eggs cysts number after First third day of treatment	efficiency	Eggs cysts number after First fourth day of treatment	efficiency	Eggs cysts number after First fifth day of treatment	efficiency
Nitazoxanie con.1	7180 b	16.7%	4200 b	52.5%	2260 B	53.1%	3180 b	54.7%	700 B	90.5%
Nitazoxanie con.2	6460 c	25.0%	2140 cd	75.8%	1180 C	75.5%	540 c	92.3%	80 c	98.9%
Nitazoxanie con.3	5560 d	35.4%	2360 c	73.3%	1080 Cd	77.5%	420 c	94.0%	60 c	99.1%
Probiotic con.3	6640 c	22.9%	1740 d	80.3%	920 D	80.9%	180 d	97.4%	40 c	99.4%
Positive control	8620 a		8860 a		4820 A		7020 a		7400 a	

Different characters in one column mean significant differences at probability level 0.05

While the first concentration of nitazoxanide has decreased the number of parasite eggs on the first, second and third day, as follows: 7180, 4200, 2260 egg cysts / mg, but on the fourth day of treatment, the number of bags increased to 3180 cysts as efficiency rate / 53.7% and decreased on the fifth day to 700 cysts of eggs / mg and at an efficiency rate of 90.5%, the increase in the number of bags of eggs during the period of treatment is called relapse after treatment and this is supported by al- Rifai (Citation *et al*,2015) because resistance shown by parasites when the inefficiency of the dose used when activated others have a subsequent injury and the phenomenon of self-healing is not observed, which has been recorded by several researchers and may be attributed to the immunity of the host as well as auto infection, which plays an important role in the continuity of infection and increase the number of eggs cysts raised.

#### RBC count

Table (2) shows the effect of nitazoxanide and probiotic in the treatment of diarrheal cases in male rabbits on the blood picture after infection.

The results showed that there were no significant differences in the number of RBC between the animal groups treatment with nitazoxanide (I and III) and the probiotic dose compared with the control group which were  $4.837 \times L / 109$ . The above groups were  $4.825, 4.712 \times L / 109$  for the first and third concentrations of nitazoxanide. The second concentration was significantly different from the  $4.597 \times L / 109$ . The RBC population in the treated rabbits

group was  $4.727 \times L / 109$ , which was not different from the negative control group, but differed from the infection group formed the highest increase in the RBC count. concentration of hemoglobin (Hb).

As shown in Table (2), the level of hemoglobin Hb did not differ significantly in all the groups of rabbits used in the study, except for the infection group has increased blood hemoglobin significantly, where the level of hemoglobin Hb in the control group was 12.273 g /dl. The level of Hb increased to 13.442 g / dL in infection group, and when the animals were similar to recovery, it was as in the negative control group. The levels in the three concentrations of the drug and the probiotic were 12.890, 12,251, 12.385 and 12,281, respectively.

Table (2) Changes in total red blood cell count, hemoclobin ratio and volume of compressed red blood cells in rabbits treated with different concentrations of nitazoxanide and biochemist

	Total RBC count $\times 10^9$			Hb g/dl			PCV %		
	mean	±	SD	mean	±	SD	mean	±	SD
control	4.837 b	±	0.507	12.273 C	±	0.320	38.409 c	±	0.487
Parasite group	5.782 a	±	0.439	13.442 A	±	0.521	40.976 a	±	0.920
Nitazoxanie con.1	4.825 b	±	0.467	12.890 B	±	0.720	38.776 b	±	0.832
Nitazoxanie con.2	4.597 c	±	0.539	12.251 C	±	0.475	38.371 c	±	0.345
Nitazoxanie con.3	4.712 bc	±	0.524	12.385 C	±	0.466	38.505 bc	±	0.977
probiotic	4.727 bc	±	0.422	12.281 C	±	0.486	38.496 c	±	0.816

Different letters in one column mean significant differences at probability level 0.05.

#### Packed cell volume P.C.V

Table (2) also shows the PCV for control and infection groups and all treatment groups. PCV in the control group was 38,409%, and the infection rate increased to 40.976%. PCV decreased at the end of the treatment period in the three concentrations of nitazoxanide with 38.776% in the first concentration. The PCV in the other two concentrations of the drug was 38.371% and 38.505% and did not differ significantly from the negative control group, but differed from the infection group, and finally A probiotic group was (38.496%), which is statistically similar to the first and second concentration groups of nitazoxanide.

The results of the present study were agreed with a study (Al-rifai,2009) which stated that there were no significant differences in hemoglobin Hb, RBC and PCV between the extract and the treatment of spiramycin between the concentrations used in the experiment. There was also no significant difference between Control and treatment groups. The values remained at the normal level compared with the negative control group. These results were not consistent with the study (salem,2015). For both PCV and Hb in Microsporidiosis patients, the rates were normal and not consistent with (Abdulsadah *et al*,2013) Children who suffer of the infection with hidden spores and that they suffer from anemia. The differences between the results may be due to the duration of infection. The parasite may be chronic for months. Side effects such as loss of fluids from the body due to diarrhea are associated with anorexia leading to malnutrition. This leads to a decrease in the amount of Hb and PCV, it was observed in Experimental rabbits that there was no significant different between treatment groups and control, it is also shown that this parasite does not affect physiologically on both PCV, Hb, and RBC.

The reason for these high results in treatment rabbits is due to the drought of infected animals due to a decrease in fluid volume in the body leading to an increase in the concentration of Hb, PCV and RBC (Al-samarrai,1999).

Total and differential count of white blood cells:

#### *WBC*

The results of the present study showed a difference in the total number of white blood cells (Table 3). The statistical analysis showed significant differences ( $P < 0.05$ ). There was a decrease in the total number of white blood cells of the positive group compared to the negative group. The number of white blood cells in the negative rabbit group was  $8.174 \times 10^9 / L$  and the number decreased to  $7.174 \times 10^9 / L$  when rabbits were infected with the parasite. There was no significant difference in the infection group in the nitazoxanide treated rabbits for the three concentrations, which were 7.158, 7.345, and  $7.337 \times 10^9 / L$ , respectively but the height number was in the group treatment with the probiotic where the WBC number was  $8.137 \times 10^9 / L$ , which is not significantly different from WBC in the negative control group.

The reduction of the parasite may be due to the fact that the white blood cells are rapidly stimulated when the antigen enters, leading to large numbers of migration to the location of the parasite by the adsorbents that are attracted to them. So the reduction of WBC number to half can be noticed, because it can kill and destroy the pathogenic organisms as the molecules resulting from the break-up can stimulate T cells and thus stimulate the acquired immune system. (Feldman *et al*,2002) As for the increase in the total number of white blood cells in the group of rabbits treated with probiotic, these results confirm the effect of lactic acid bacteria in raising the immune response to the body compared to the two sets of antibody, which decreased the number of lymphocytes, which indicate an increase in production of T & B cells (Schnare *et al*,2001).

#### *Lymphocyte*

As shown in Table (3), we observe the significant differences in the lymphocyte count of all the groups under the study. The most significant feature is the decrease in the number of lymphocytes in the positive control group (infected) of  $1.914 \times 10^9 / L$ . Negative control was  $3.393 \times 10^9 / L$ . When rabbits treated with nitazoxanide at the binging, the numbers of lymphocyte increased. The numbers in the second and third concentrations of the drug reached  $3.357$  and  $3.308 \times 10^9 / L$ . They did not differ significantly from the number of lymphocyte in the negative control group, And from the results in this table also is increase the number of lymphocytes in the rabbit probiotic treatment group compared to all treatments where the number reached to  $4.334 \times 10^9 / L$ . This result was agreed with (Valeur *et al*,2004) which reached an increase in WBC and lymphocytes in probiotic rabbits.

The reason for the reduction in the numbers of lymphocytes in animals infected by parasite is due to the phenomenon of vascular dilation of lymphocytes. And may cause the decline of numbers of lymphocytes around the blood vessels, but these cells do not have the ability to phagocytosis, but have a specific ability to the movement of amoebic and in most circumstances these cells out of the blood vessel but near it, so the role of lymphocytes in inflammation is to contribute to the immune response Leading to the secretion of toxic substances and the formation of antibodies that have a direct and effect against the parasite (Al-saadi,2010). The change in the differential count of white blood cells may indicate antibody production (Roitt *et al*,1998)

Table (3) Changes in total and differential CBC rates in rabbits treated with different concentrations of nitazoxanide and bioenergy

control	Total WBC			Lymphocyte $\times 10^9/L$			granulocyte $\times 10^9/L$			Monocyte		
	mean	$\pm$	SD	mean	$\pm$	SD	mean	$\pm$	SD	mean	$\pm$	SD
Parasite group	8.174 a	$\pm$	0.531	3.393 b	$\pm$	0.370	4.059 a	$\pm$	0.402	1.344 b	$\pm$	0.115
Nitazoxanie con.1	7.174 b	$\pm$	0.556	1.914 d	$\pm$	0.205	2.935 b	$\pm$	0.509	0.795 b	$\pm$	0.317
Nitazoxanie con.2	7.158 b	$\pm$	0.577	2.732 c	$\pm$	0.526	3.291 b	$\pm$	0.501	0.888 b	$\pm$	0.545
Nitazoxanie con.3	7.345 b	$\pm$	0.488	3.357 b	$\pm$	0.695	2.916 b	$\pm$	0.635	1.067 b	$\pm$	0.586
probiotic	7.337 b	$\pm$	0.439	3.308 b	$\pm$	0.306	3.307 b	$\pm$	0.619	1.219 b	$\pm$	0.290
	8.137 a	$\pm$	0.599	4.334 a	$\pm$	0.517	4.332 a	$\pm$	0.634	1.519 a	$\pm$	0.609

The current results have agreed with (Thongsong *et al*,2008) the rise of lymphocytes in animals treated with bacterial probiotic and their filtration. The role of natural microorganisms enhances the role of flora in the defense of the body by preventing the proliferation of harmful bacteria, as well as stimulating the action of natural and acquired immunity, and this is confirmed by (Rastall &Gibson,2002) in his study, he pointed out that the use of microbial preparations increases the effectiveness of the immune system in terms of Stimulating the humoral and cellular immunity together in the chicken used in its study.

Granulocyte cells of WBC (acidophil, basophil, neutrophil)

The results obtained in the present study, as shown in Table (3), showed a significant decrease in the control group of granulocyte counts in the infedted and nitazoxanide groups of the three concentrations, with a significant difference at a probability level of 0.05. The infection group was  $2.935 \times 10^9 / L$ , In the first, second and third groups of nitazoxanide, 3.291, 2.916 and  $3.307 \times 10^9 / L$ , respectively, the number of granulocyte cells was statistically similar to the negative control group at  $4.332 \times 10^9 / L$ . this Results corresponding with a study of (Al-Saedi,2004) decrease a white blood cells and granulocytes and lymphocytes in infected animals with cryptosporidium parasite and the absence of significant differences between infected animals and Nitazoxanide treatment animals.

The cause of the reduction of the granulocytes in the blood after the entry of the parasite is to the migration of these cells to the location of parasite, which leads to the decline and eliminate it by the process of phagocytosis, so this cell is an important part of the phagocytic system (Hamdy,2017).

The most important cells that contribute to the phagocytosis process are neutrophils, and the characteristics of the infection are cell resurfacing (janssen,1997). acidophil have a significant role in the immune response to parasitic infections by migrating to infection sites due to the Eosinophil chemotactic factor, are highly interacting to remove granulocytes from mast cells. These parasitic cells also attack by binding to immunoglobulin IgG and IgE, which causes the loss of their granules and the release of their contents. (janssen,1997).

The acidophil destroy the parasites through their enzymes, which affects the outer wall of the parasite, which leads to its destruction. The acidophil that help in its function are mast cells. IgE binds to the receptors on the mast cells when exposed to the parasite and attracts to acidophil to the site of infection, and the cells of the current act to stimulate inflammation at the site of the concentration of the antigen and work similar to the mast cells (Roitt *et al*,1998)

### Monocyte cells (mast cell)

According to the results in the Table ( 3 ) The number of Monocyte in rabbits was decreased in the group of infection. However, according to the statistical analysis, the decrease was not significant for the control group at 0.795%, while the treated group of nitazoxanide in the three concentrations was not significantly different from the Negative control group, and reached 0.888, 1.067 and 1,219% in the three concentrations, respectively.

While the number of rabbits treated with biochemist was quite different. Monocyte cells increased after completion of the treatment and the number reached 1.519%. This result is significantly different from the negative control group at the probability level of 0.05. The results of this study were consistent with the results of a decrease in the number of whole and differential white blood cells in rabbits infected with *Cryptosporidium parvum*. The reduction in the number of single cells in the parasite-infected animals may be due to the migration of these cells from peripheral blood to infection sites because they are non- The important quality against many of the infections, and their transformation into macrophage cells (Madigan *et al*,2003) as these cells contribute to the phagocytosis to eliminate the parasite. From the observation of the blood picture of white blood cells, we can point to the role of lactic acid spores in stimulating the immune system of rabbits in the case of immunization and treatment with these probiotic compared to the antibiotic(Levinson & jawetz,2000).

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