Effect of non-protein amino acids β-amino butyric acid (BABA) on the intestinal G- bacterial community

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Abstract

This study was conducted in the animal house of the College of Education for Girls, to investigate the effect of beta-amino butyric acid (BABA) on the microorganisms in the digestive system of mice. The bacteria present in the stool were isolated and diagnosed. And that intestinal bacteria have an effective role in influencing human health. However, few factors that affect the bacteria in the digestive system are known. Despite the increase in the number of microbial communities colonizing the gastrointestinal tract, many of these bacteria have been found to possess virulence factors that can negatively affect the host organism and protect it from prevailing antibiotics. BABA) was used with four treatments (100, 200, 300, 0) mg/kg animal weight denoted by A, B, C, and D. Male rats were immersed orally in four doses per week, and the results showed that the amino acid effectively affected On the intestinal bacterial community, where the third concentration of the fourth dose gave the lowest rate of the number of bacterial isolates, which led to the elimination of a large number of them, followed by the second concentration and then the first compared to the comparison treatment that achieved the highest rate of the number of isolates. While histidine had no negative effect on the Toll-Like Receptor 4 (TLR4), the results showed that TLR4 was a normal receptor.

Introduction

The digestive system of humans and animals is the main host for different microbes, as it contains a large number of pathogenic and non-pathogenic microbes. Intestinal microbes begin to form immediately after birth and are affected by the type of nutrition and environmental factors, as well as their important role in metabolism, as well as their active role in important metabolic functions in the fermentation of complex carbohydrates that cross from the digestive process, various substances consumed in the diet, fats, amino acids and proteins, breakdown of bile acids, absorption of vitamin K and many components of vitamin B, the community of bacteria that colonizes the host intestine is closely related to the various physiological functions of the host, including Digestion, nutrient metabolism, and immunity, and may also influence the host's adaptation to the environment and evolution, therefore, the intestinal bacterial community has an important role in maintaining human life and health (Ilhan, 2018; Oliphant and Allen-Vercoe, 2019; Fülling et al., 2020). Oliver et al., (2021) reported that dietary shifts have a direct effect on the gut microbiome through a selective selection of microbes capable of utilizing different nutrients. Bogatyrev et al., (2020) also mentioned in their study that the digestive system plays a prominent role in human physiology as a primary site for enzymatic digestion and nutrient absorption, and the revival of the small intestine has been involved in various human diseases, such as non-alcoholic steatohepatitis and inflammatory bowel diseases. Gut bacteria exploit the nutrients provided by the host organism, on the other hand, the host uses many products of gut bacteria metabolism as essential material for ATP production in the colon, causing bacterial metabolites to leak from the gut into the bloodstream and interfere with the host’s cellular bioenergetics mechanism (Tomasova et al., 2021). The diverse microbial community inhabiting the human gut possesses a broad metabolic repertoire, so the gut microbiota is a major factor in shaping the biochemical profile of the diet, and thus its impact on host health and disease

(Rowland et al., 2018). Rivera-Piza and Lee, (2020) note that the gut microbiota may induce metabolic diseases, such as obesity, through genetic and environmental factors and pathways linking metabolism to the immune system. And that the gut microbiota can affect the absorption of nutrients and energy storage, and thus the prevalence of obesity and metabolic disorders (Bogatyrev et al., 2020).

β-amino butyric acid (BABA) is one of the rare free compounds found in nature. It has been known to stimulate plant resistance against various pathogens such as viruses, bacteria, fungi, and worms (Prieto et al., 2021; Hegedus et al., 2022). One such promising molecule is β-aminobutyric acid (BABA), which modulates the defence system at the molecular level (Choudhary et al., 2021). Parker et al., (2021) stated that BABA has plausibly non-biological and non-terrestrial origins. β-amino butyric acid (BABA) is one of the rare free compounds found in nature. It has been known to stimulate plant resistance against various pathogens such as viruses, bacteria, fungi, and worms (Baccelli et al., 2017). Ma et al., (2020) indicated in their study that b-aminobutyric acid (BABA), which is an environmentally friendly agent, can be widely used to induce plant resistance to biotic and abiotic stress, and the results showed that adding 0.2 mM of BABA led to reduce the damage caused to plants by the effects of stress and to preserve the cell structure. Treatment with β-aminobutyric acid (BABA) increased the plants' resistance and protective ability against bacterial infections such as wheat leaf rust (*Puccinia triticina*) disease caused by Pseudomonas protegens (Bellameche et al., 2021). Yu et al., (2022) reported that BABA limits the reproduction of nematodes. BABA also has resistance against the necrotic fungi *Rhizopus stolonifer* (Li et al., 2021). A male (Sagheer and Jasim, 2020) in his study on rats showed that B-amino butyric acid (BABA) affected some immune variables of male Sprague Dawley rats infected with the bacteria pseudomonas aeruginosa.

Monitor-like receptors (TLRs) are essential immune receptors with the ability to recognize conserved molecular patterns expressed by pathogens and damaged cells (Kashani et al., 2021). Activation of TLRs can lead to a range of effects including inflammatory responses, cell cycle modification, apoptosis, or regulation of cell metabolism (Akesolo et al., 2022; Fitzgerald and Kagan, 2020). TLRs are an evolutionarily ancient family of pattern recognition receptors (Fitzgerald and Kagan, 2020). Activation of these receptors is essential for the regulation and sustainability of the inflammatory response and sensitivity to danger signals. The TLR family consists of 10 known receptors, and there is some evidence to suggest that each is expressed within human platelets, These receptors are essential for coordinating and sustaining the inflammatory response to both types of danger signals (intrinsic and extrinsic) and initiating a cascade of molecular events that lead to the initiation of autoimmunity (Hally et al., 2020).

The monitoring-like receptor (TLR-4) belongs to the family of TLR which, when activated in cells of the body, initiates a chain of events (Mohamed et al., 2022). Toll-like receptor-4 is the primary receptor for LPS and is involved in the proinflammatory response, and Toll-like-4 is expressed by different types of cells throughout the human body (Ducharme et al., 2022). TLR-4 can be considered an important target for the treatment of febrile seizures in FS rats (Zaniani et al., 2022). SARS-CoV-2-induced myocarditis and multiorgan infection may be due to TLR4 activation and aberrant TLR4 signalling and hyperinflammation in COVID-19 patients TLR4 contributes significantly to SARS-CoV-2 pathogenesis, and its overactivation

also leads to a prolonged or excessive innate immune response. This makes TLR4 a promising therapeutic target in COVID-19 (Aboudounya and Heads, 2021). Activation of TLR4 signalling upregulates several pro-inflammatory cytokines and chemokines, leading to nephritis. Therefore targeting TLR4 and its downstream effectors could be an effective therapeutic intervention to prevent renal inflammation and subsequent kidney damage (Jha et al., 2021).

Sagiyan, (2010) mentioned in his study that the addition of non-protein amino acid in the formulation of drugs leads to the prolongation of the effect of drugs and maintains their efficacy, and through the development in chemistry and modern biology, the non-protein amino acid (NPAAs) has become a powerful tool for the development of peptide-based drugs. Song et al., (2021) also mentioned in their study that the introduction of α-N-methylated histidine into the peptides can improve their biological activities, membrane permeability and proteolytic stability. In a study conducted by Al-Kubaisi, (2020) on the effects of β-amino butyric acid (BABA) on male rats with type 2 diabetes, the concentration of 100 used led to positive physiological and biochemical changes in the serum of male diabetic rats, progressively with higher concentrations. This indicates that increasing the concentration gave better effects, and this is what was worked on in this study by giving a higher concentration of acid. Beta amino acids such as beta-aminobutyric acid (BABA) can predispose plants to resistance to many diseases (Tao et al. 2022).

BABA will be a useful treatment for diabetes by improving glucose metabolism and restoring oxidative stress, and may also have antioxidant properties. Also, BABA was effective in reducing DNA damage in the livers of diabetic mice (Thamir and Jasim, 2021).

Bacteria have recently shown resistance to antibiotics, prompting researchers to search for more effective antibiotics, especially anti-bacterial, which are among the most important pathogens around the world, and in some cases can be considered the main cause of important diseases, mostly due to their ability to produce many Among the virulence factors that enable it to penetrate body tissues, generate infection and resist antibiotics (Jalil et al., 2017). Given the active role that non-protein amino acid plays in various vital and abiotic processes, this study aims to know the role played by β-amino butyric acid (BABA) in influencing microbes in the digestive system.

Materials And Methods

Experience design

In the experiment, 20 male Sprague Dawley rats were used, which were prepared from the National Center for Drug Control at the age of 10-12 weeks, with weights ranging between (210-160) g. The animals were placed in cages for rats in the animal house of the College of Education for Girls - University of Anbar. Suitable environmental conditions were created for the animals, including ventilation and temperature ranging between (28-25) C and appropriate lighting periods of up to 12 hours/day, then they underwent a period of printing for 10 days to acclimatize to the surrounding environment before starting the stages of the experiment. Experimental animals were distributed into four groups, each group containing five

animals that were dosed with different concentrations of 100, 200, and 300 mg/kg for groups A, B and C, respectively, while group D was without addition (control treatment), The animals were dosed in stages, T1 = the first dose, T2 = the second dose, T3 = the third dose, T4 = the fourth dose.

Preparation of a non-proteinogenic BABA solution

The histidine solution was prepared by dissolving it in 10 ml of distilled water for each of the four groups and according to the concentrations indicated against each of them. Then the required doses were prepared for each animal according to weight and dosed weekly for four weeks using a gavage syringe (Perret-Gentil, 2010).

Collecting samples

Samples of the faeces of male rats were taken before and after each stage of dosing and transferred to the laboratory for the necessary tests.

Isolation and identification of bacteria

Bacterial isolates were diagnosed by growing them on culture media with MacConkey agar and Nutrient agar and then conducting phenotypic and biochemical tests on them.

Phenotypic tests

In these tests, the phenotypic characteristics of the colony growing on the culture media were based, on the colony's shape, colour, edge shape, height from the surface, its texture, in addition to the characteristics of the phenotypic cells in terms of shape, size, arrangement, interaction with Gram dye under the light microscope (MacFaddin). , 2000).

Biochemical tests

A series of biochemical tests were carried out in the Research Laboratory / College of Education for Girls on bacterial colonies isolated from the faeces of male rats after the necessary dilutions were made, including the Catalase test, Oxidase test, Indol Production test, Methyl red test, Fox- Voges - Proskauer (VP), Citrate utilization test, urease test Starch hydrolysis test, Motility test, Gelatin liquefaction test, Nitrate reduction test and Hydrogen sulfide production test.

Examination of bacterial isolates with VITEK®2

The bacterial isolates under study were diagnosed using the VITEK®2 device for verification. The examination was carried out using the 64-hole D-GNB Kit containing 41 tests, according to the manufacturer's instructions, as the cards contain 18 assays for sugars representation, 18 tests for sugars fermentation, and two tests for decarboxylase In addition to three various tests (urease test, exploitation of malonates, tryptophan deaminase)", using a vacuum device, then vaccinating the cards using the

suspended microorganism under examination, then inserting the cards into the device. Fluorescence is measured every 15 minutes and the results are determined after 3 hours (Dina and Rania, 2014).

Prepare the PCR mixture:

25 µL of the PCR reaction consisted of a green master mix (Promega), primer, deionized water and DNA template with the following volume of PCR mixture used in the study listed in Table (1).

Table: (1) The polymerase chain reaction mixture used in the current study.

NO	Content of reaction mixture	The volume of thereaction mixture for a single tube.
1	Green master mix	12.5 µL
2	DNA template	5 µL
3	Forward primer(10 Picomol)	1µL
4	Reverse primer(10 Picomol)	1µL
5	Nuclease free water	5.5µl
	Total volume	25 µl

DNA loading and electrophoresis:

5 µl was mixed with 2 µl loading dye. Samples were carefully loaded into individual gel pits, and then electrical power was turned on at 70 V/cm. for 1 hour. The beams stained with ethidium bromide in the gel were photographed using UV light-365 nm.

How Toll-Like Receptor 4 (TLR4) Gene Detection Works

Detection of the Toll-Like Receptor 4 (TLR4) gene was performed using special amplification primers. A portion of the DNA was amplified using a forward primer (ACTGGGTGAGAAACGAGCTG') as well as a reverse sequence primer (CAGCAATGGCTACACCAGGA) primer kit provided by IDT (Integrated DNA Technologies, Canada). PCR amplification was performed in total by polymerase chain reaction amplification to detect a gene (Table 2).

Table 2: PCR amplification program for TLR4. gene detection

Gene	Step	Temperature(^{0}C)	Time	No.of cycle
large	Initial denaturation	94	4 min	1
	Denaturation	94	45 sec	35
	Annealing	62.2	1 min	30
	Extension	72	1 min	
	Final extension	72	7 min	1
	Hold Temperature		-	

Results And Discussion

The results of the statistical analysis showed a clear difference in the number of isolates, although the effect was not high for the first and second concentrations of acid (Fig. 3). The control treatment (D) gave the highest average number of bacterial isolates, which amounted to 93, 90, 88, 83 and 80, while The third concentration (C) gave lower rates of 64, 68, 52, 61 and 69 for *E. coli*, *Proteus mirabilis*, *Proteus penneri*, *Proteus hauseri* and *Enterobacter gergoviae*, respectively, while concentrations A and B showed less effect on the number of bacterial isolates compared to at concentration (C), this indicates the effective effect of beta-amino butyric acid (BABA), as some types of bacteria showed weak resistance against acid, which led to a decrease in the number of isolates, while there were few significant differences between the first and second concentrations of acid. This was indicated by Al-Tai et al. (2021) in their study on the effect of acid on staphylococcus bacteria, where they concluded that β-amino butyric acid (BABA) has a positive role in bacterial resistance.

The results (Fig. 4) indicated that the second dose showed a significant difference in the number of bacterial isolates, where the third concentration (C) of the dose gave the lowest average number of isolates compared to the control treatment, which gave the highest rate of the number of bacterial isolates, and this indicates the effective effect of Beta-amino butyric acid (BABA) on the ability of bacteria to resist and survive in the presence of an acid, which reduces their numbers almost inversely with the increase in acid concentration. The third concentration affected more than the first (A) and the second (B) concentrations on the number of bacterial isolates, which in turn gave lower rates of the number of bacteria The isolates were compared to the control treatment, and the concentrations in the second dose (T2) were more effective and effective in several bacterial isolates than the concentrations in the first dose (T1), and the three concentrations in the third (T3) and fourth (T4) stages of dosing also led to the disappearance of some bacterial species and were more effective with an increase in concentration and this shows the importance of giving acid to the animal's Several doses and not given in one dose. This is consistent with what Asif et al. (2021) indicated that β-aminobutyric acid (BABA) severely inhibits the proliferation of microorganisms.

The results of the statistical analysis (Fig. 5) showed that the third dose showed a significant difference in the number of bacterial isolates, where the third concentration of the dose gave the lowest average number of isolates, followed by the second concentration and then the first concentration compared to the control treatment, which gave the highest rate of the number of isolates, and this shows the effective role of Beta- amino butyric acid (BABA) in reducing the ability of bacteria to resist and perhaps the acid acted as an anti-bacterial in the digestive system of rats, which led to a clear reduction in their numbers and the disappearance of some types with an increase in acid concentration. The data also indicated that the three concentrations of acid in the third dose (T3) had a role Effective in affecting bacteria more than the first (T1) and second doses (T2), and this shows the importance of giving animals several doses and not giving acid in one dose. This is consistent with the results obtained by Chalupowicz et al., (2021) in their study of the effect of β-aminobutyric acid (BABA) on bacteria, where they found that BABA inhibits the multiplication of Salmonella enterica.

The results of the statistical analysis (Fig. 6) showed that the fourth dose showed a significant difference in the number of bacterial isolates, whereas the control treatment gave the highest rate of the number of bacterial isolates while adding acid to the three concentrations (first, second and third) significantly affected the number of bacterial isolates, as it did not lead to a decrease The number of bacterial isolates only, but led to the disappearance of a large number of bacterial species, especially at the third concentration of the dosing. Bacteria that have disappeared at this stage of dosing are *E. coli, Pseudomonas auroginosa, Burkholderia cepacia, Salmonella diarizonae,* and *Klebsiella pneumonia*. The three concentrations of the fourth dose also achieved lower rates of the number of bacterial isolates and the disappearance of some species compared to the other concentrations of the first, second and third doses, and some also disappeared. This is consistent with what was indicated by Gur et al., (2021); Choudhary et al., (2021) showed that non-proteinogenic β-aminobutyric acid (BABA)) showed resistance against probiotics.

Effect of Beta-amino butyric acid (BABA) on Toll-like 4 receptors

It is clear from the results of the study (Picture 5) that β-aminobutyric acid (BABA) in the four dosing stages A, B, C and D was given to male Sprague Dawley rats at different concentrations of 100, 200 and 300 mg/kg had a positive role in maintaining On the Toll-like 4 receptor, this may, in turn, reduce or inhibit molecules involved in inflammatory processes (such as cytokines, chemokines, prostaglandins, complement factors, and cell adhesion molecules). Where it was found that the receptor Toll-like 4 was not negatively affected by the increase in the concentration of the amino acid (BABA) and it maintained its natural image. It is even believed that it had activated and stimulated and thus increased the resistance and immunity of the animals used in the experiment, and this was observed through the increase in their weight and behavior As well as increasing the quantities of food consumed compared to the control treatment (without adding BABA). This indicates the role of Toll-like 4 in regulating the organism's defence against infection. This was indicated by Akesolo et al., (2022) in their study that activating the receptors leads to enhancing the immunity and resistance of organisms against many diseases that can infect them in certain environmental conditions.

Conclusions

The results obtained in this study showed that the non-proteinogenic amino acid BABA affected the gram-negative intestinal bacterial community, as biochemical tests showed that the number of bacterial isolates decreased significantly with the increase in the concentration of BABA. In the fourth dose, a large number of bacteria disappeared, and this indicates the possibility of using BABA as an antibiotic against a large number of bacteria. Also, the amino acid led to the maintenance of Toll-like 4 naturally, as well as its stimulation and activation, even with high concentrations, and this reflected positively on the increase in the immunity of the animals used in the study.

Declarations

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Statement of Animal Ethics

We declare that animals received appropriate treatment upon a study which was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the University of Anbar (Date.22/6/2022/ No. 80).

Conflict of Interest Statement:

The authors declare that this article hasn't any conflict of interest. The authors have no relevant financial or non-financial interests to disclose.

Data Availability Statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Mohammed A. Jasim contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Eman Ahmed Mikhlif, Mohammed. A. Jasim. The first draft of the manuscript was written by Eman Ahmed Mikhlif and corrected by Mohammed. A. Jasim. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

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Figures

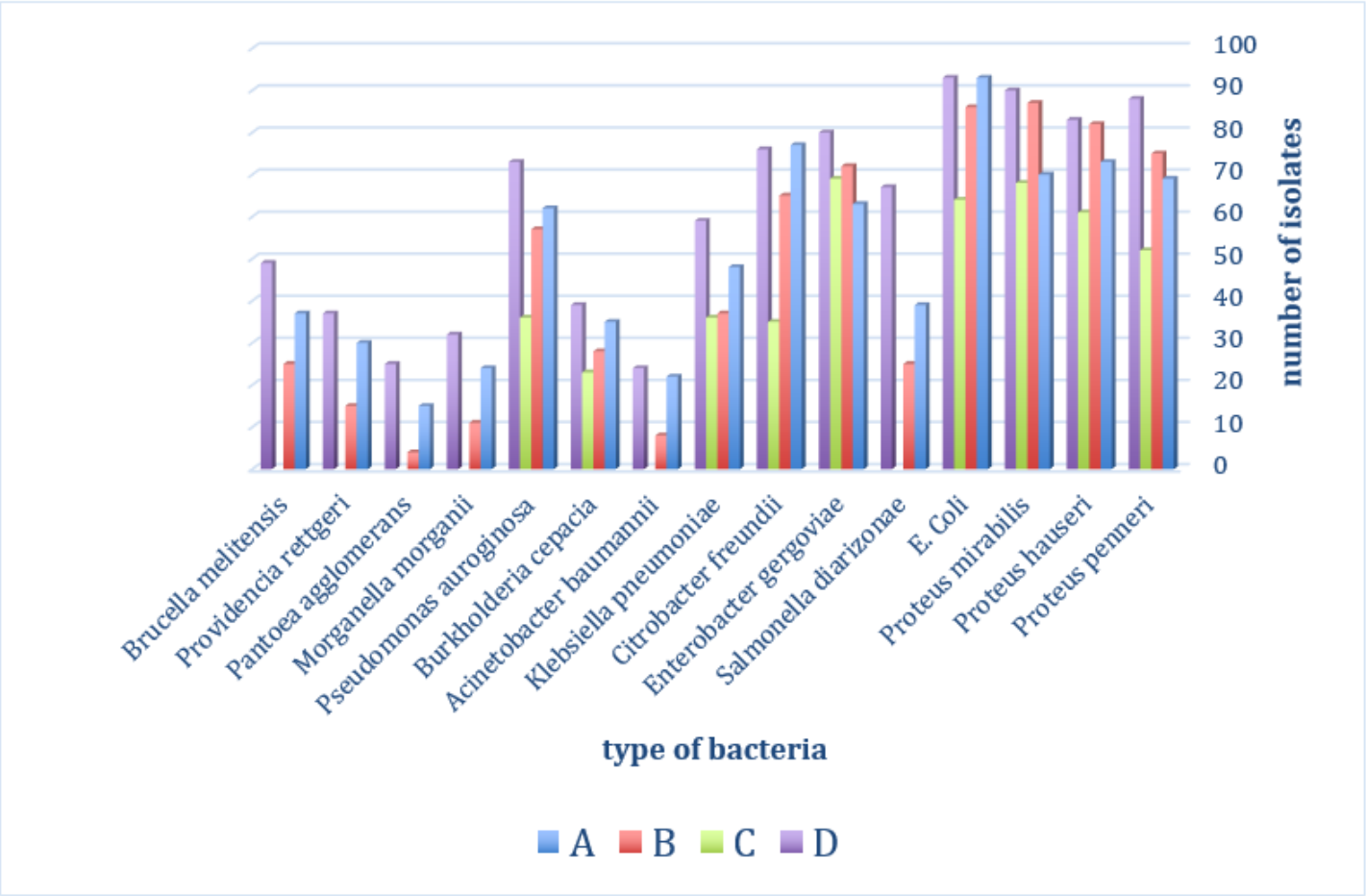

Figure 1

Figure (3) Effect of Beta-amino butyric acid (BABA)) on the type and number of intestinal bacterial isolates after the first dose

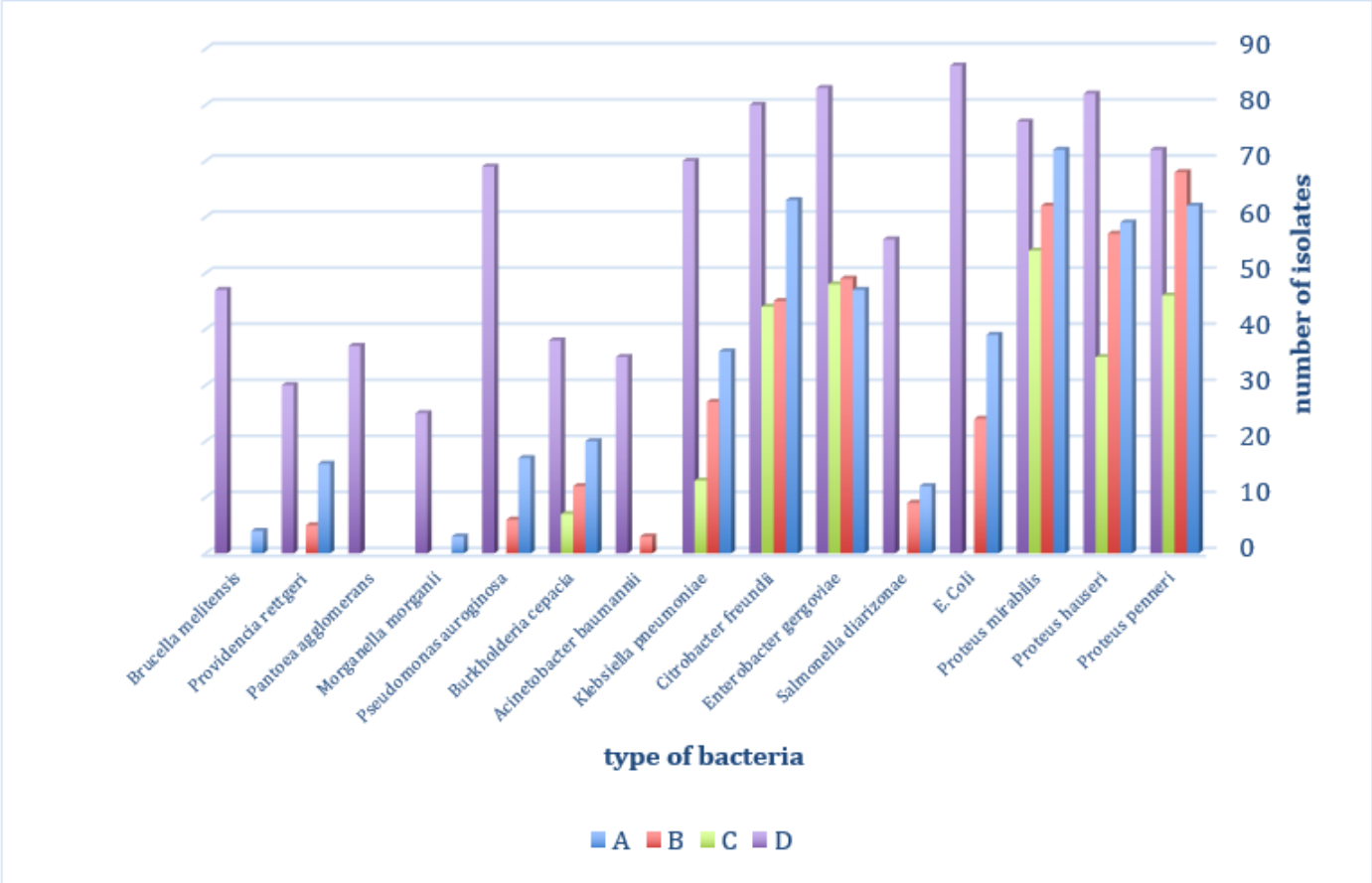

Figure 2

Figure (4) Effect of Beta-amino butyric acid (BABA)) on the type and number of intestinal bacterial isolates after the second dose

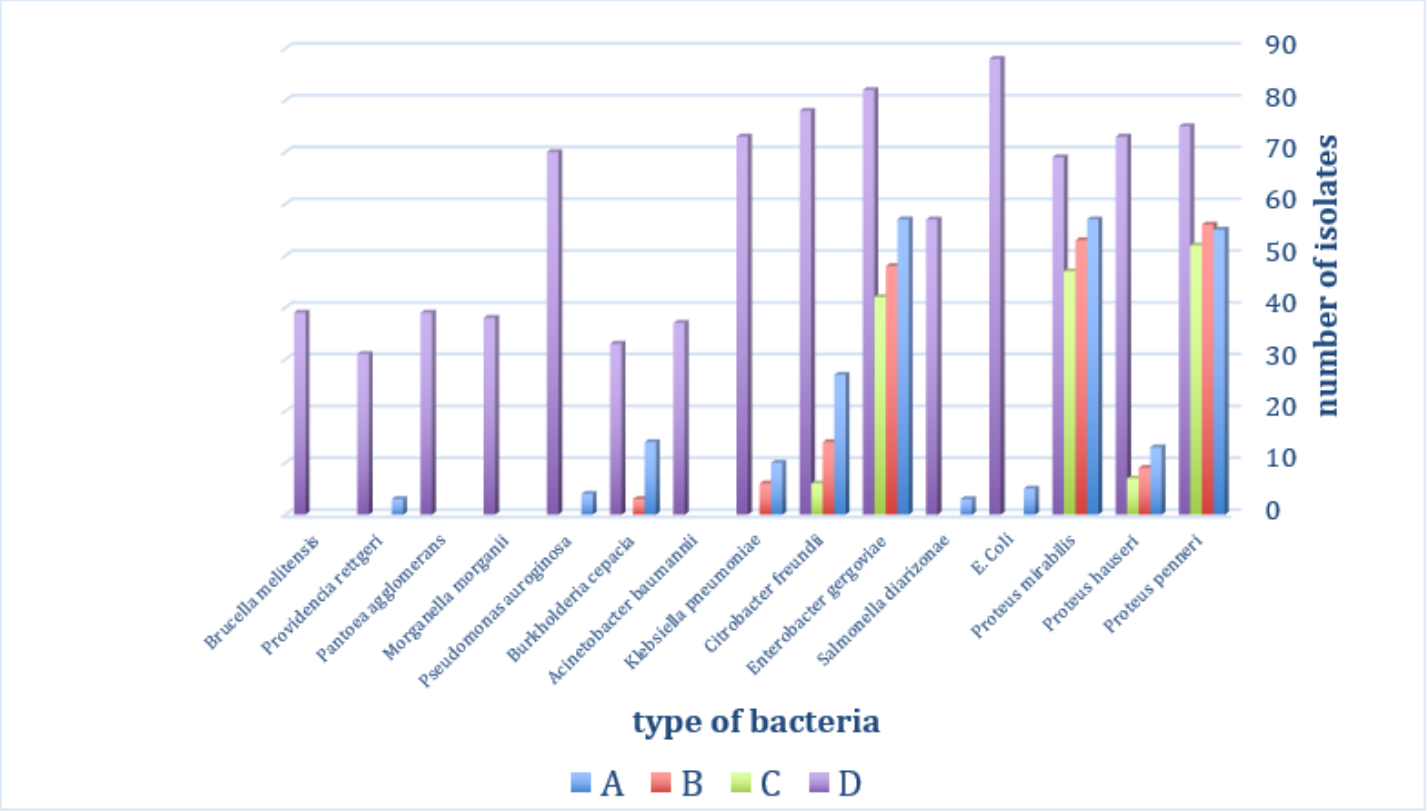

Figure 3

Figure (5) Effect of Beta-amino butyric acid (BABA)) on the type and number of intestinal bacterial isolates after the third dose

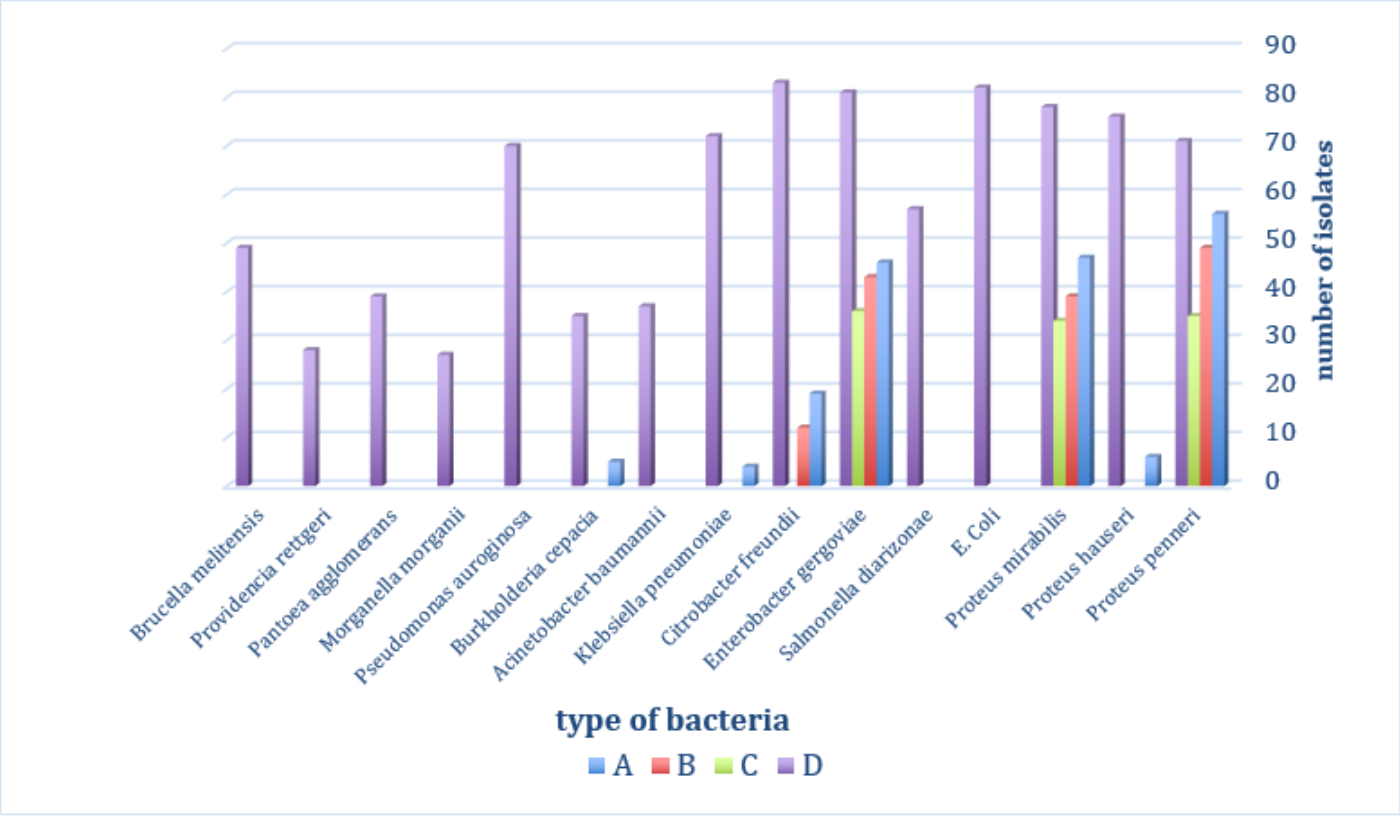

Figure 4

Figure (6) Effect of Beta-amino butyric acid (BABA) on the type and number of intestinal bacterial isolates after the fourth dose