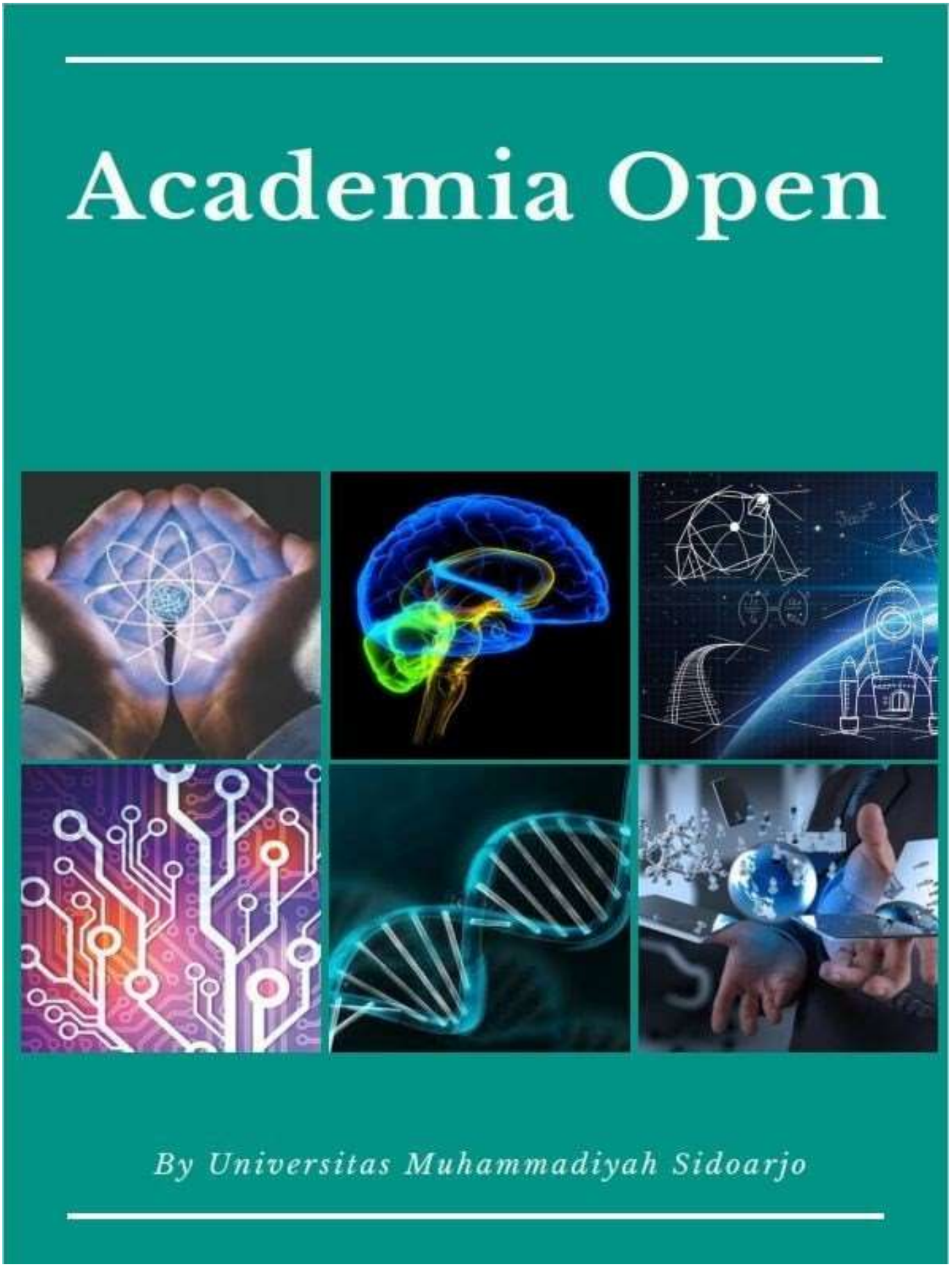
Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13267



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DOI: 10.21070/acopen.11.2026.13267

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Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13267

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ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13267

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Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13267

Lactoferrin on Physiological Characteristics and Intestinal Microorganisms in Anemic Rats

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Abstract

General Background: Iron is essential for hematopoiesis, immune competence, and growth, and its deficiency leads to anemia with systemic consequences. **Specific Background:** Lactoferrin, an iron-binding glycoprotein, exhibits hematopoietic, immunomodulatory, and antimicrobial activities that may benefit anemia and gut microbiota balance. **Knowledge Gap:** Experimental evidence comparing purified and commercial lactoferrin on hematological indices and intestinal microbial populations in anemic models remains limited. **Aims:** This study evaluated the effects of purified and commercial lactoferrin on blood parameters and gut microorganisms in anemic female rats. **Results:** Lactoferrin administration increased red blood cells, hemoglobin, and platelets, reduced white blood cell counts, enhanced lactic acid bacteria, and decreased coliforms, Staphylococcus spp., and Enterococcus spp., with strongest effects at 30 µg/kg purified lactoferrin, while Salmonella showed minimal change. **Novelty:** The study demonstrates dose-dependent superiority of purified lactoferrin over commercial forms. **Implications:** Purified lactoferrin represents a promising dietary supplement for anemia management and gut health modulation.

Keywords : Lactoferrin, Iron Deficiency Anemia, Hematological Parameters, Gut Microbiota Modulation, Female Albino Rats

Highlight :

- Purified supplementation significantly increased erythrocyte, hemoglobin, and platelet indices in anemic female models.
- Dose-dependent treatment reduced leukocyte counts, indicating pronounced immunomodulatory activity.
- Beneficial gut bacteria increased, while coliforms, staphylococci, and enterococci declined after intervention.

Published date: 2026-01-05

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13267

Introduction

Numerous vital biological processes, such as oxygen transport via hemoglobin, cellular metabolism and various redox reactions, require iron, an essential trace element [1]. Dietary iron deficiency significantly affects general physiological functions and biochemical processes, ultimately leading to anemia [2][3]. Specifically, anemia occurs when the body does not have enough iron to make hemoglobin an important protein in red blood cells that is responsible for transporting oxygen to tissues and organs [4]. Pregnant women and young children are particularly susceptible to anemia due to their high iron needs. Seriously Plus studies show a high prevalence of anemia in low-income countries often exacerbated by malnutrition and poverty with an estimated 40% of children under the age of six suffering from the condition [5][6].

Lactoferrin (LF), a 70 kDa multifunctional glycoprotein belonging to the transferrin family, plays an important role in biological systems. It has a strong affinity for iron ions (Fe^{3+}), binding two of these ions per molecule. This protein is naturally present in colostrum and breast milk, as well as in polymorphonuclear white blood cells (WBCs) and endocrine secretions of various mammals, such as saliva, tears and bronchial mucosa. The biological activities of lactoferrin are diverse and include immune enhancement, direct interaction with bacterial cell walls, and potent antiviral, anti-parasitic and anti-inflammatory properties. Recently, its potential role in the prevention and treatment of cancer has also been proven [7].

Lactoferrin's broad-spectrum antimicrobial effect is primarily due to its ability to bind iron, thus depriving pathogenic microorganisms such as *E. coli*, *Salmonella* and *Staphylococcus aureus* of these nutrients essential for their growth and survival. In addition to its ability to bind iron, lactoferrin (LF) can directly interact with microbial cell walls, compromising their integrity and leading to cell death. It also has significant antiviral activity against a variety of viruses, including influenza viruses, herpes viruses and immunodeficiency virus human. Its antifungal properties help prevent the growth of fungi such as *Candida albicans* and are also effective against parasites such as *Toxoplasma gondii, Trypanosoma cruzi* and *Plasmodium falciparum*.

The aim of the study, given the role of iron in homeostasis, immunomodulation and antimicrobial defense and the study was to determine the therapeutic effectiveness of LF in alleviating anemia in female albino rats.

Materials and Methods

Lactoferrin Preparation

Purified LF was produced in vitro according to the methodology described by Daher [8]. The experimental lactoferrin solutions were formulated at concentrations of 10, 20 and 30 µg/kg, which were called T3, T4 and T5 treatments, respectively. These pure preparations were compared to the commercially available LF product T6. Seriously, all LF formulations were then administered to laboratory animals suffering from induced anemia.

Experimental Animals and Husbandry

This study was carried out in the Animal House of the College of Science, Kufa University. Seriously, a total of 42 adult female albino rats, approximately 1 month old and weighing between 125 and 160 g, were obtained from the above facility. Upon arrival, the animals underwent a two-week acclimatization period to ensure they were healthy and disease-free.

Rats were housed in plastic cages with metal mesh lids and 500 mL water bottles with metal teats. There were four animals in each cage. Cages were lined with sawdust bedding that was changed three times per week to maintain cleanliness throughout the study. The animals were kept under controlled temperature conditions and under the supervision of veterinary staff. During the experiment, they received commercial protein pellets and tap water ad libitum.

Experimental Design

The 42 female albino rats were randomly divided into six equal groups of seven rats each. The animals were subjected to the following feeding regimen for 30 days:

1. T1 (non-anemic control): rats were fed with normal food without inducing anemia.
2. T2 (Anti-Anemia): Anemic rats were induced and fed with a normal diet.
3. T3: Anemic rats received purified lactoferrin at a concentration of 10 µg/kg/day.
4. T4: Anemic rats received purified lactoferrin at a concentration of 20 µg/kg/day.
5. T5: Anemic rats received purified lactoferrin at a concentration of 30 µg/kg/day.
6. T6: Anemic rats received commercial lactoferrin.

Anemia Induction

The anemia was caused by a perforation of the heart that means a direct withdrawal of blood from the heart. Initial complete blood counts (CBC) were performed on a subset of rats using an animal-specific blood analyzer. Five days after blood collection, a second CBC test was performed on the same group to confirm that the induction of anemia was successful and yielded a positive result [9].

Sample Collection and Physiological Parameter Assessment

Thirty days after the end of the treatment period, the animals were humanely sacrificed after anesthesia with chloroform. Blood samples were taken by intracardiac puncture into anticoagulant gel tubes to obtain serum for subsequent post-dose analysis of blood markers. The following physiological parameters were assessed:

1. Red Blood Cell (RBC) count: Performed according to Math [10].
2. Total Hemoglobin (Hb) concentration: Estimated following the methodology by Quinlivan [11].

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3. Total platelet count (PLT): determined with a blood cell counter using ammonium oxalate solution as diluent. Specifically, 0.02 mL of blood was added to 0.38 mL of ammonium oxalate solution in a clean test tube, mixed, and allowed to stand for 15 min to stabilize the cell. Platelets were then counted in five small squares of the large central square at 40x magnification, calculated as follows:

Platelet count (cells/mm^3) = Number of cells counted×100

1. Total and Differential White Blood Cell (WBC) count: Blood smears were prepared, air-dried, and stained with Leishman Stain dye (Annex 3). Differential counts of granular and non-granular white blood cells were performed under an oil immersion lens [12]. Briefly, 8 drops of Leishman dye covered the smear for 2 minutes, followed by 16 drops of phosphate buffer solution, mixed well, and left for 10 minutes. Slides were then rinsed with distilled water and air-dried. One hundred white blood cells were counted and their differential type was expressed as a percentage. The total WBC count was calculated as follows:

WBC/mm ^{3}= (Number of cells counted) × 50.

Isolation and Quantification of Fecal Bacteria

After a 15-day acclimatization period, on the 7th day of the experiment, stool samples were taken from the rats to evaluate the viability and isolation of the bacteria. Fresh stool samples were serially diluted tenfold in saline and inoculated into different selective culture media.

Selective Media for Microorganism Growth:

1. MRS agar: prepared by dissolving 70 g of medium in 1 liter of distilled water, adjusting the pH to 7, and autoclaving at 121°C for 15 minutes at 1 atmosphere. This method is used for *Lactobacillus* spp. was used to isolate and count (8 and manufacturer's instructions).
2. MacConkey agar: dissolve 52 g of solid culture medium in 1 liter of distilled water, then heat to boiling for one minute, then sterilize. This medium was used to enumerate coliform bacteria (manufacturer's instructions).
3. Salmonella Shigella (SS) agar: prepared by dissolving 36 g of solid medium in 1 liter of distilled water heating to boiling for 1 minute and then autoclaving. This medium was used to measure Salmonella bacteria (according to the manufacturer's instructions).
    a. Mannitol salt agar (MSA): prepared by dissolving 111.02 g of medium in 1 liter of distilled water, heating for 1 minute, and then autoclaving. Seriously, this medium was used to quantify staphylococcal bacteria (manufacturer's instructions).
    b. Bile Esculin Azide Agar (BEA): it's prepared by dissolving 63.5 g of medium in 1 liter of distilled water and sterilizing it in an autoclave. This medium was used to enumerate enterococcal bacteria (according to the manufacturer's instructions).

Ethical Statement

The necessary ethics are approved by the Ethics Committee of the College of Agriculture/ Karbala University on the acceptance number- UOK. Agri. No. 4.2025.

Results

Effect of Lactoferrin on Blood Parameters

Fig. 1 shows a significant increase in the number of RBC in all lactoferrin-treated groups (T3, T4, T5, and T6) compared to the control group. Specifically, RBC counts were 6.242, 6.77, 8.816, and 8.104 × 10^6/µL for T3, T4, T5 and T6, respectively, whereas the control group exhibited an RBC count of 6.072 × 10^6/µL, also showed Fig (1).

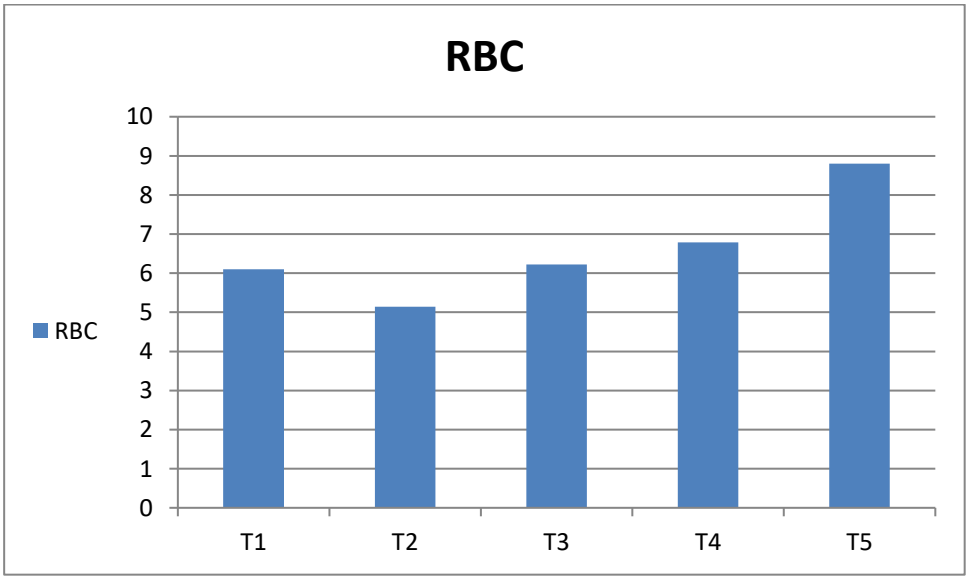

Figure 1: Effect of LF on red blood cell counts in treated animals

This figure shows the average red blood cell count of the animals in the different treatment groups: T1: control (non-anemic) group, T2: anemic control group, T3: LF at 10 µg/kg/day, T4: LF at 20 µg/kg/day, T5: LF at 30 µg/kg/day, T6: commercial LF.

The results of the study also showed the effect of LF on Hb levels. The T5 treatment showed a clear and statistically significant increase in hemoglobin reaching 16.10 g/dL which was significantly higher than the other treatments. The T3 and T4 treatments also showed higher

hemoglobin levels 15.232 g/dL and 15.890 g/dL respectively. In comparison the hemoglobin level in the control treatment was 14.90 g/dL while in the T6 (commercial lactoferrin) treatment it was 15.298 g/dL. These results are presented visually in Fig.2 and also showed Fig 3.

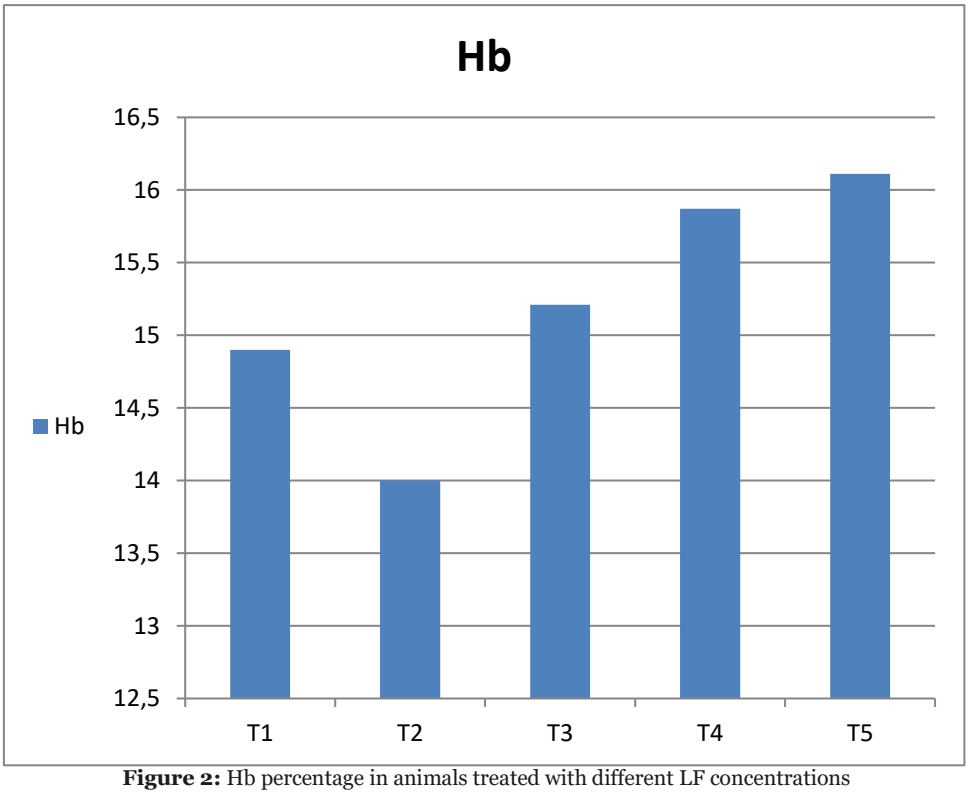

Figure 2: Hb percentage in animals treated with different LF concentrations

This figure shows the percentage of mean Hb in animals in the different treatment groups: T1: control (non-anemic) group, T2: anemic control group, T3: LF at 10 µg/kg/day, T4: LF at 20 µg/kg/day, T5: LF at 30 µg/kg/day, T6: commercial LF.

Effect of LF on Platelet Count

The results presented in Fig. 3 demonstrate the effect of LF on the PLT count. Increased PLT counts were observed for T3, T4 and T5 treatments at 363.8, 386.8 and 398.4 × 10^3/µl respectively. In contrast, the PLT number in the control treatment was 348.6 × 10^3/µl. Seriously commercial LF treatment (T6) showed a PLT count of 373.6 × 10^3/µl, also showed table (1).

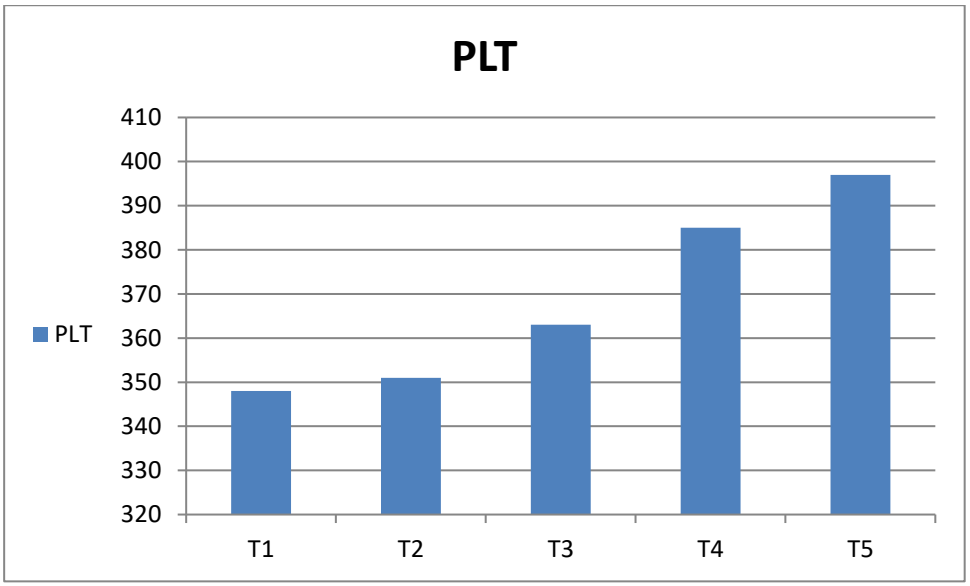

Figure 3: Platelet count in animals treated with different concentrations of lactoferrin

This figure shows the mean PLT count of the animals in the different treatment groups: T1: control (non-anemic) group, T2: anemic control group, T3: LF at 10 µg/kg/day, T4: LF at 20 µg/kg/day, T5: LF at 30 µg/kg/day, T6: commercial LF.

Effect of LF on White Blood Cell Counts

Furthermore, as shown in Fig. 4, the WBC count was significantly changed in all LF -treated groups showing a significant decrease. Specifically, the WBC count was significantly reduced during T5 treatment to 9.992 x 10^3/µl. Both T3 and T4 treatments showed low numbers reaching 10.206

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DOI: 10.21070/acopen.11.2026.13267

x 10^3/µl and 10.056 x 10^3/µl respectively. These values were lower than in the T6 treatment (10.050 x 10^3/µl) and significantly lower than the WBC count of the control treatment of 12.590 x 10^3/µl, as showed in table (1).

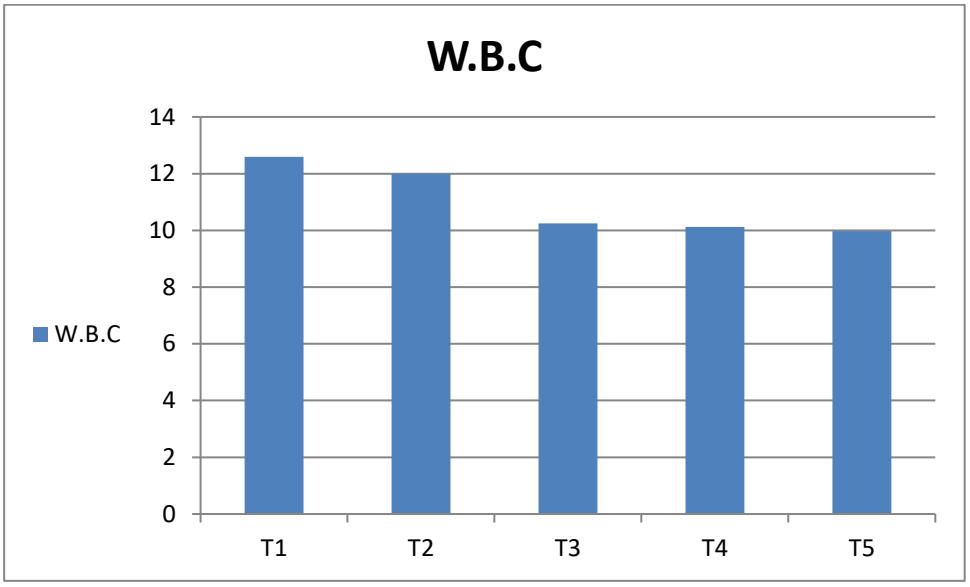

Figure 4: Effect of LF on white blood cell counts in treated animals

This figure shows the mean WBC count of the animals in the different treatment groups:T1: control (non-anemic) group, T2: anemic control group, T3: LF at 10 µg/kg/day, T4: LF at 20 µg/kg/day, T5: LF at 30 µg/kg/day, T6: commercial LF.

Table 1. Effect different concentrations of LF in female rats on RBC, Hb, PLT and WBC

groups	Means ± S.E			
	RBC 10^6/µL	**Hb g/dL**	**PLT × 10^3/µl**	**WBC (10^3/µl)**
T1	6.072 ± 0.05 E	14.900 ± 0.01 E	348.600 ± 1.02 F	12.590 ± 0.01 A
T2	5.148 ± 0.01 F	13.986 ± 0.02 F	352.800 ± 1.82 E	12.00 ± 0.03 B
T3	6.242 ± 0.05 D	15.232 ± 1.77 D	363.800 ± 0.01 D	10.206 ± 0.05 C
T4	6.770 ± 0.01 C	15.890 ±0.01 B	386.800± 0.86 B	10.056 ± 0.04 D
T5	8.816 ± 0.01 A	16.100 ± 0.05 A	398.400 ± 0.50 A	9.992± 0.04 D
T6	8.104 ± 0.009 B	15.298 ± 0.01 B	373.600 ± 1.80 C	10.050 ± 0.04 D

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Academia Open
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DOI: 10.21070/acopen.11.2026.13267

L.S.D 0.05	**0.0750**	**0.0564**	**4.0933**	**0.1252**

* **P value Significant ≤ 0.05**

Effect of LF on Fecal Bacterial Populations

Table (2) presents the results concerning the fecal bacterial populations. The data indicate an increase in Lactic Acid Bacteria (LAB) counts isolated from the feces of female rats treated with varying concentrations of purified LF. Specifically, the LAB count for treatment T5 was 10.83 × 10^5 cfu/mL, while treatments T3 and T4 showed counts of 8.52 × 10^5 cfu/mL and 6 × 10^5 cfu/mL, respectively. These values were higher compared to the control treatment's LAB count of 4.91 × 10^5 cfu/mL, and treatment T6 recorded 8.50 × 10^5 cfu/mL.

Conversely, the study results revealed a decline in the number of coliform bacteria. Their counts decreased in treatments T5, T4 and T3 to 5.47, 6 and 6.69 × 10^5 cfu/mL, respectively. This was a reduction compared to the control treatment (T1), which had a coliform count of 7.28 × 10^5 cfu/mL.

On the other hand, *Salmonella* showed only a minimal change in their numbers across all treatment groups when compared to the other bacterial species investigated.

Table 2. Effect of different concentrations of LF on number of bacteria in rats with anemia after 30 days.

Treatment	Lactic Acid bacteria	Coliform	*Enterococcus*	*staphylococcus*	*Salmonella*
T1	4.91	7.28	8.68	7.62	6.60
T2	4.31	8.93	8.98	7.72	6.69
T3	6	6.69	7.68	4.62	6.45
T4	8.52	6	7.21	4.31	6.32
T5	10.83	5.47	7.04	4.00	6.20
T6	8.50	5.93	7.1	4.28	6.35

Bacteria are counted according to the logarithm of the number 10^5 the unit of measurement is cfu/ml

The table further indicates a decrease in the abundance of *Staphylococcus* spp. in the treatment groups. Specifically, counts for treatments T5, T4 and T3 were 4.00, 4.31and 4.62 × 10^5 cfu/mL, respectively. This contrasts with the T1 control group, which had a *Staphylococcus* spp. count of 7.62 × 10^5 cfu/mL.

Moreover, the number of *Enterococcus* spp. bacteria also decreased in treatments T5 (7.04 × 10^5 cfu/mL), T4 (7.68 × 10^5 cfu/mL), and T3 (7.21 × 10^5 cfu/mL). Treatment T6 also showed a reduced count of 7.1 × 10^5 cfu/mL, compared to T1 which had 8.68 × 10^5 cfu/mL.

Discussion

LF influences iron absorption and possesses significant immunomodulatory properties. More and more research is highlighting the role of lactoferrin in increasing intestinal iron absorption, although the exact mechanisms behind the effects are not yet fully understood. A meta-analysis of 11 studies including 1262 participants showed that daily oral LF supplementation resulted in greater improvements in blood iron serum ferritin and Hb levels than oral ferrous sulfate. However, partial iron absorption was found to be better than ferrous sulfate [13].

In addition to its general effect on iron absorption LF has a significant effect on various blood parameters. Studies show that taking LF results in significant improvements in markers such as Hb, mean corpuscular volume (MCV), serum iron transferrin saturation (TS) and serum ferritin. At the same time a significant decrease in total iron binding capacity (TIBC) was observed. it's worth noting that LF increases transferrin saturation and serum ferritin more efficiently than iron sulfate. These results are consistent with previous research in adult populations that reported that lactoferrin supplementation significantly increased Hb blood iron and blood ferritin levels compared with conventional oral iron supplementation [14][15].

In addition to its documented effects on blood parameters LF also has various antimicrobial properties. While its ability to bind iron, thus making it inaccessible to gut bacteria, partially explains its antimicrobial functions, LF uses a bunch of other mechanisms to exert its effects [16].

Despite its powerful iron-binding capacity, the exact role of LF in the intestinal absorption of iron is the subject of ongoing debate. Although a significant proportion of iron in breast milk is bound to LF, the relatively high concentration of LF combined with the low total iron content of breast milk suggests that only a small fraction (<3%) of LF binds efficiently to iron in vivo [17]. Furthermore, while LF is prone to degradation in the stomach, a significant portion survives gastric transit in adults. Survival rates are likely higher in infants due to the less acidic stomach environment [18].

Research shows that LF has significant health benefits. For example, fortification of infant formula with LF can reduce the incidence of lower respiratory tract infections in healthy infants. Research has scientifically proven that lactoferrin effectively modulates immune and inflammatory responses and enhances iron absorption, making it a treatment for anemia in both children and adults [19]. Also, LF contributes to improving iron status and reducing the incidence of gastrointestinal diseases [20].

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The oral administration of LTF not only helps restore iron balance by increasing iron absorption but also prevents inflammatory processes that contribute to the development of chronic disease-related anemia a condition characterized by functional iron deficiency that is essential for physiological processes [21].

LF protects against infection and inflammatory complications arising from diagnostic surgical procedures in pregnant women. During pregnancy its beneficial and multifaceted effects are the inhibition of oxidative stress the normalization of the intestinal microflora and the reproductive system and the improvement of carbohydrate and fat metabolism. Plus, LF contributes to the protection of the intestinal barrier function promotes wound healing [22].

LF is a well-established factor with broad antimicrobial properties and plays a crucial role in the body's primary defense mechanism against bacteria, viruses, fungi and protozoa [23].

The primary mechanism of lactoferrin's inhibitory effect against microorganisms is its remarkable ability to bind iron. Ions effectively depriving bacteria of these nutrients necessary for growth and reproduction. Addition of exogenous iron sources beyond the chelating capacity of LF can reverse this inhibitory effect and restore bacterial growth. This mechanism of iron deficiency was first described by Arnold, who showed that human lactoferrin inhibits the growth of bacteria such as Vibrio cholerae and *Streptococcus mutans* in an iron-rich environment as well as the growth of various *Staphylococcus* species, *Listeria monocytogenes* and *Bacillus* [24]. In addition to iron chelation the antibacterial role of LF is also attributed to the co-binding site of lipopolysaccharide (LPS) which is a major component of the outer membrane of Gram-negative bacteria [25].

Farnaud and Evans further elucidated lactoferrin's antimicrobial role against pathogenic protozoa, specifically citing its efficacy against *Toxoplasma gondii*, *Trypanosoma cruzi* and *Plasmodium falciparum*. While defense mechanisms against other protozoan diseases have yet to be fully defined, it's hypothesized that the activity of LF involves a number of mechanisms, some of which remain to be identified.

The broad antimicrobial spectrum of lactoferrin can be attributed to a variety of proposed mechanisms. LF can primarily interfere with the absorption of essential iron (Fe^{3+}) by pathogenic microbes, thus inhibiting their growth [26].

LF exhibits direct antimicrobial activity through its LF derivative (LFcin). LFcin, a stable antimicrobial peptide generated from the N-terminal region of lactoferrin during pepsin digestion, can directly bind to the membrane surface of pathogenic microbes. This effect disrupts microbial integrity and is effective against bacteria viruses' fungi and protozoa. Notably LFcin has greater antibacterial activity than LF itself.

The bioavailability of iron bound to iron-saturated lactoferrin (Fe-LF) remains a subject of active debate. One prevailing hypothesis suggests that the absorption of Fe-LF is challenging due to its low dissociation constant, that means that its strong binding to iron prevents iron absorption. In contrast, an alternative perspective suggests that Fe-LF is more easily absorbed, largely due to the presence of lactoferrin-specific receptors identified in the gut. This persistent discrepancy in understanding is likely due to variability from several experimental factors, including the specific dose of iron ingested, the degree of iron saturation with lactoferrin, the source of lactoferrin itself, the animal model used in the study, and the presence of other dietary components that may influence the results [27].

The exact bioavailability of iron bound to iron-saturated lactoferrin (Fe-LF) remains a matter of considerable scientific debate. One prominent theory is that the low dissociation constant of Fe-LF makes it difficult to absorb because its strong binding to iron prevents iron from being released for cellular uptake. On the other hand, an alternative view suggests that Fe-LF is more easily absorbed a phenomenon perhaps facilitated by the presence of LF-specific receptors in the intestinal lumen. While ferrous salts, such as ferrous sulfate are traditionally known to be better absorbed than other iron compounds, the identification of specific LF receptors at the brush border of human and monkey enterocytes, antiques iron-saturated lactoferrin (Fe-LF) can be more than that .Iron salts are easily absorbed [28].

To test this hypothesis, the researchers sought to determine the iron dose threshold at that iron-deficient mice could recover from anemia when treated with Fe-LF but not iron salts. In a preliminary study, Kawakami administered 0-200 µg of iron per day orally as ferrous sulfate or Fe-LF to iron-deficient mice. Seriously, Changes in Hb levels between days 0 and 28 were then analyzed to determine this threshold dose. Anemic mice were then given this specific dose of iron orally to evaluate whether the lactoferrin-derived iron showed better absorption.

Zhao suggested that the anti-inflammatory effects of LF may partially explain its superior efficacy in improving iron status, even though it exhibits a lower fractional iron absorption (FIA) compared to ferrous sulfate. This phenomenon is likely because increased levels of inflammatory cytokines impair dietary iron absorption and mobilization from the reticuloendothelial system, which is critical for meeting the hematopoietic demands of red blood cell production, largely due to increased hepcidin synthesis.

Second reducing inflammation may directly contribute to increased erythropoiesis. Inflammatory cytokines especially interleukin 6 (IL-6) are known to inhibit this vital process [29][30][31].

The mechanism of action of LF in modulating the cellular immune response in an in vivo model of inflammation is multifaceted. Leukogram analyses consistently demonstrated a regulatory role for LF in the immune system, consistent with the observations of Ward and Conneely [32]. These researchers reported that LF has the unique ability to up- or down-regulate immune system activity within organisms, a dynamic effect that positions it as a preventive and therapeutic agent. Furthermore, the protective effect of LF against trauma-induced damage may be attributed to its ability to modulate the relative balance between inflammatory and anti-inflammatory mediators [33]. For example, Farid reported that administration of LF resulted in a general inhibition of monocytes and macrophages in the context of protection against carbon tetrachloride (CCl_4)-induced injury [34]. This inactivation was characterized by a significant decrease in pro- and anti-inflammatory mediators. Importantly, significant reductions in pro-inflammatory mediators, such as interleukin-1 beta (IL-1β) and anti-inflammatory mediators, such as interleukin-10 (IL-10) and paraxonase 1 (PON1) were observed when a prophylactic or therapeutic administration protocol was used simultaneously, indicating an anti-inflammatory state [35].

LF actively contributes to the modulation of the inflammatory response by affecting the dynamics of white blood cells, especially neutrophils. It facilitates their recruitment and accumulation at the site of tissue injury, thereby enhancing immune defense against microbial invasion. This effect promotes the closure of wounds and tissue gaps by stimulating interactions between cells that ultimately speed up the healing process [36].

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Conclusion

As described in the attached study data, the results show that lactoferrin supplementation, especially when given in a pure form and at higher doses, has a transparent and reproducible therapeutic effect on anemia and gut microflora of female rats. The purified lactoferrin produced a significant improvement in the major hematological indices, such as the red blood cell count, hemoglobin concentration, and platelet levels, whereas the elevated cavitary white blood cell count was also reduced, suggesting both hematopoietic support and immunomodulatory activity by the purified lactoferrin. Concurrently, lactoferrin positively altered the gut microbiota by promoting beneficial lactic acid bacteria and inhibiting potentially pathogenic genera including coliforms, Staphylococcus spp., and Enterococcus spp. with the strongest effects being observed at the 30 μg/kg dose, while Salmonella populations largely remained unaffected. Both effects are dose-dependent and substantiate the benefits of using purified lactoferrin over commercial preparations, presumably by virtue of higher bioactivity and a superior ability to bind iron. These findings have implications in the developing lactoferrin as a functional food supplement or adjunct treatment for inflammation- or gut dysbiosis-associated anemia. Further studies are thus needed to clarify the actual molecular events regulating iron availability, immune response and microbiota modulation, and to assess the long-term effects, optimal dose, translation in other animal models and in humans.

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