"Synergistic Effects of Colocasia esculenta, Spinacia oleracea, and Yogurt as a Synbiotic Combination on Hemoglobin Levels, Body Weight Reduction, and Clotting Time: An Experimental Study in a Mice Model."

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APPROVAL

This research project, entitled ***"Synergistic Effects of Colocasia esculenta, Spinacia oleracea, and Yogurt as a Synbiotic Combination on Hemoglobin Levels, Body Weight Reduction, and Clotting Time: An Experimental Study in a Mice Model"***, submitted by ID: 232-46-030 from the Department of Pharmacy, Daffodil International University, has been evaluated and approved as meeting the standards for the partial fulfillment of the requirements for the degree of Master of Pharmacy (M. Pharm). The project has been deemed satisfactory in terms of its academic rigor, scientific contribution, and adherence to the prescribed format and content guidelines.

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DECLARATION

I, Israt Jahan Tamanna, hereby declare that this thesis work entitled ***"Synergistic Effects of Colocasia esculenta, Spinacia oleracea, and Yogurt as a Synbiotic Combination on Hemoglobin Levels, Body Weight Reduction, and Clotting Time: An Experimental Study in a Mice Model"*** has been carried out through my efforts and facts arrived at my observation under the supervision of Professor Dr. Sharifa Sultana, Associate Professor and Associate Head of the Department of Pharmacy, Daffodil International University, for the partial fulfillment of the requirements for the degree of Master of Pharmacy (M. Pharm). I also declare that this work has not been previously submitted, in whole or in part, to any other university or institute for the award of any other degree or professional qualification except as specified.

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DEDICATION

This work is a heartfelt tribute to my cherished parents, whose love and guidance have been my foundation; my inspiring supervisor, whose wisdom and support have been my compass; and my incredible friends, whose encouragement and belief in me have been a source of endless motivation. Their unwavering presence has not only shaped this project but also enriched my journey, for which I am profoundly grateful.

"Synergistic Effects of Colocasia esculenta, Spinacia oleracea, and Yogurt as a Synbiotic Combination on Hemoglobin Levels, Body Weight Reduction, and Clotting Time: An Experimental Study in a Mice Model."

Abstract

Iron deficiency and metabolic imbalances remain significant global health concerns, often exacerbated by limited iron bioavailability in plant-based diets due to inhibitors like phytates and oxalates. This study evaluates the efficacy of a synbiotic combination of *Colocasia esculenta* (Taro), *Spinacia oleracea* (Spinach), and yogurt in improving hemoglobin levels, regulating body weight, and enhancing clotting time in a non-anemic mice model. The primary aim was to assess the impact of the synbiotic combination on hemoglobin levels, body weight, and clotting time, and to compare its performance against standalone plant-based interventions and synthetic supplementation. Six groups of mice (Control, Colocasia, Spinach, Probiotic [yogurt], Treatment [synbiotic combination], and Standard [synthetic iron supplement]) were monitored for 14 days. Key parameters—hemoglobin levels, body weight changes, and clotting time—were measured. Statistical analysis, including ANOVA and post-hoc tests, was conducted to determine group differences. The Treatment Group achieved the highest hemoglobin levels (16.75 g/dL, $F = 11.18$, $p < 0.0001$), significant weight reduction (-5.50 g, $F = 28.76$, $p < 0.0001$), and the shortest clotting time (32.75 seconds, $F = 11.18$, $p < 0.0001$). The synbiotic combination demonstrated superior efficacy compared to standalone interventions and synthetic supplementation, emphasizing the role of probiotics in enhancing non-heme iron absorption, improving metabolic efficiency, and supporting systemic health. This study underscores the potential of a synbiotic combination of *Colocasia esculenta*, *Spinacia oleracea*, and yogurt to improve hematological and metabolic parameters. The findings suggest that such dietary strategies offer sustainable and effective alternatives to synthetic supplementation. Future research should focus on isolating bioactive compounds, understanding their mechanisms of action, and validating these results through human clinical trials.

Keywords: *Colocasia esculenta*, *Spinacia oleracea*, yogurt, hemoglobin, body weight, clotting time, synbiotic, iron bioavailability.

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CHAPTER-1

INTRODUCTION

1.1. Introduction

Hemoglobin is a vital protein responsible for oxygen transport, and its synthesis is closely linked to iron availability in the diet. Suboptimal iron intake and utilization can negatively impact hemoglobin levels, even in non-anemic individuals, leading to reduced metabolic efficiency and overall health [1][2]. Iron bioavailability depends significantly on its dietary source. Heme iron from animal-based foods is highly bioavailable, while non-heme iron from plant-based foods is less readily absorbed due to the presence of inhibitors such as phytates and oxalates [3] [4]. For populations consuming predominantly plant-based diets, addressing the limitations of non-heme iron absorption is crucial. Emerging strategies to enhance non-heme iron absorption include the use of probiotics and synbiotic interventions. Probiotics, such as Lactobacillus and Bifidobacterium species, can modify the gut microbiota, enhance intestinal health, and improve the bioavailability of minerals, including iron [5] [6].

In this study, two iron-rich plant sources, Colocasia esculenta (taro) and Spinacia oleracea (spinach), are evaluated for their effects on hemoglobin levels. Colocasia esculenta contains significant iron and micronutrients, including vitamin C, which may aid iron absorption [7]. Spinacia oleracea, known for its high iron content and folate, is essential for red blood cell production and maintaining healthy hemoglobin levels [8]. However, both Colocasia esculenta and Spinacia oleracea contain phytates and oxalates, which can hinder iron absorption, limiting their effectiveness as sources of bioavailable iron [9][10][11].

Yogurt, a natural source of probiotics, can potentially enhance iron absorption by modifying gut microbiota and supporting nutrient uptake. Probiotics in yogurt, including Lactobacillus and Bifidobacterium species, improve intestinal health by lowering gut pH, promoting beneficial bacteria, and strengthening the gut barrier, which can increase mineral bioavailability [12]. The combination of yogurt with prebiotic-rich plant sources like Colocasia esculenta (taro) and Spinacia oleracea (spinach) may potentially enhance iron absorption by reducing the inhibitory effects of phytates and oxalates. Studies suggest that yogurt, through its fermentation process and probiotic content, can help mitigate the negative impact of these compounds on iron bioavailability. In one study, the addition of yogurt to raw purslane leaves significantly reduced the soluble oxalate content of the mixture, indicating yogurt's potential in lowering oxalates in plant foods. Similarly, when yogurt, coconut milk, or coconut cream were added to raw leaves, the overall oxalate content was reduced by simple dilution, suggesting that yogurt can play a role in improving the bioavailability of nutrients like iron. By reducing the oxalate content, the combination of yogurt with plants like spinach and taro may help promote higher iron absorption, potentially leading to increased hemoglobin levels [13].

While the effects of Colocasia esculenta, Spinacia oleracea, and probiotics on hemoglobin and iron absorption have been studied individually, the potential synergistic impact of combining these components has not been widely explored. It is hypothesized that a dietary intervention that includes Colocasia esculenta, Spinacia oleracea, and yogurt will yield greater benefits in hemoglobin levels than any component individually. Such a combination could serve as an accessible nutritional strategy for populations at risk of iron deficiency, particularly those on predominantly plant-based diets [14].

Emerging research highlights the role of gut microbiota in modulating iron absorption and overall nutrient bioavailability. Probiotic bacteria such as Lactobacillus and Bifidobacterium have been shown to secrete organic acids that lower gut pH, enhancing the solubility and absorption of non-heme iron [15]. Furthermore, these probiotics can inhibit the growth of pathogenic bacteria that compete for iron, thereby indirectly increasing its availability for the host [16].

Plant-based iron sources, despite their limitations, offer a range of bioactive compounds that may contribute to hematological health. Spinach contains folate, an essential nutrient for red blood cell production, while Colocasia is rich in vitamin C, a known enhancer of iron absorption [17] [18] [19]. The combination of these plant sources with yogurt-based probiotics creates a unique dietary synergy that warrants detailed investigation.

In addition to hematological effects, dietary interventions can also influence metabolic parameters such as body weight. Probiotics have been implicated in modulating gut microbiota composition, which in turn affects energy metabolism and nutrient absorption [20]. Studies suggest that probiotics may promote weight regulation by enhancing fatty acid metabolism and reducing low-grade inflammation associated with obesity [21]. However, the role of synbiotic combinations in this context remains understudied.

Clotting time, another important physiological parameter, provides insights into the hemostatic balance of an organism. Nutrients like iron and folate play critical roles in maintaining hemostasis by supporting red blood cell and may have the effect on platelet function [22]. While synthetic supplements can address specific deficiencies, natural dietary interventions may offer a broader range of systemic benefits.

This research aims to explore the synergistic effects of Colocasia esculenta, Spinacia oleracea, and yogurt on hemoglobin levels, body weight, and clotting time in a non-anemic mice model. By assessing these parameters, the study provides insights into the potential of natural synbiotic combinations as sustainable strategies for improving hematological and metabolic health, particularly in populations with limited access to animal-based foods or synthetic supplements.

The findings of this study are expected to contribute to the growing body of evidence supporting the use of functional foods and synbiotic interventions to address nutritional deficiencies and promote systemic health.

1.2. Plant Profile

1.2.1. *Colocasia esculenta* (Taro)

1.2.1.1. Taxonomy and Classification

- **Scientific Name**: *Colocasia esculenta (L.)*
- **Family**: Araceae
- **Common Names**: Taro, Dasheen, Eddoe, Elephant Ear
- **Origin and Distribution**: Native to Southeast Asia and India, now cultivated worldwide in tropical and subtropical regions [23].

Figure 1: *Colocasia esculenta (L.)*
Image Courtesy: Google Image

1.2.1.2. Morphology

- **Growth Habit**: Herbaceous perennial plant with large, heart-shaped leaves.
- **Stem**: Underground corms are starchy and used as a staple food in many regions.
- **Leaves**: Long-petioled leaves with broad blades and prominent veins.
- **Flowers**: Rarely flowers in cultivation; inflorescence consists of a spathe and spadix [17].

1.2.1.3. Cultivation and Ecology

- **Soil**: Prefers well-drained, fertile soils with a pH of 5.5–7.0.
- **Climate**: Thrives in warm, humid climates. Sensitive to frost.
- **Propagation**: Primarily by corms or cormels.
- **Pests and Diseases**: Vulnerable to taro leaf blight (*Phytophthora colocasiae*) and nematodes [24].

1.2.1.4. Phytochemical Constituents

The phytochemical analysis of Colocasia esculenta (taro) reveals a rich presence of bioactive compounds, including alkaloids, glycosides, flavonoids, terpenoids, saponins, and phenols. These constituents were identified in both aqueous and methanolic extracts of the plant's leaves and tubers. The study suggests that the abundance of these phytochemicals makes Colocasia esculenta a promising candidate for developing new pharmaceutical formulations.

Additionally, the research indicates that the methanolic extract of Colocasia esculenta showed a better extractive value compared to the aqueous extract, suggesting that the phytoconstituents are more concentrated in the methanolic extract. This finding underscores the potential of Colocasia esculenta as a staple food with significant phytochemical content, recommending its consideration in the pharmaceutical industry [25].

1.2.1.5. Health Benefits

1. **Iron Absorption**: Taro's high vitamin C content enhances the absorption of iron from plant-based sources, making it a useful food for improving iron bioavailability in vegetarian diets.
2. **Gut Health**: Taro contains resistant starch, which can have prebiotic effects, promoting the growth of beneficial gut bacteria. This, in turn, may support digestive health.
3. **Anti-inflammatory**: Taro is believed to have anti-inflammatory properties due to the presence of antioxidants and other bioactive compounds in its flesh.
4. **Rich in Fiber**: The fiber content of taro can support digestive health, improve satiety, and regulate blood sugar levels [24].

1.2.2. *Spinacia oleracea* (Spinach)

1.2.2.1. Taxonomy and Classification

- **Scientific Name**: *Spinacia oleracea* L.
- **Family**: Amaranthaceae (formerly Chenopodiaceae)
- **Common Names**: Spinach
- **Origin and Distribution**: Believed to have originated in Persia (modern-day Iran), now cultivated globally [26].

Figure 2: *Spinacia oleracea* L.
Image Courtesy: Google Image

1.2.2.2. Morphology

- **Growth Habit**: Annual or biennial leafy green vegetable.
- **Stem**: Erect, green, and tender.
- **Leaves**: Ovate to triangular leaves, smooth or crinkled (savoy types).
- **Flowers**: Small, inconspicuous greenish-yellow flowers; dioecious or monoecious [26].

1.2.2.3. Cultivation and Ecology

- **Soil**: Prefers loose, well-drained loamy soils with pH 6.0–7.5.
- **Climate**: Grows best in cool weather (16°C–24°C); bolts in heat.
- **Propagation**: Direct sowing of seeds.
- **Pests and Diseases**: Affected by downy mildew (*Peronospora farinosa*), aphids, and leaf miners [27].

1.2.2.4. Phytochemical Constituents

The phytochemical analysis of Spinacia oleracea (spinach) leaves reveals the presence of several bioactive compounds, including carbohydrates, tannins, flavonoids, terpenoids, and phenols. These constituents were identified across various solvent extracts, with methanol proving to be the most effective solvent for extracting these phytochemicals. The study also quantified the total flavonoid and phenolic content, highlighting the antioxidant potential of spinach leaves.

Additionally, spinach is known to contain a variety of flavonoids such as quercetin, myricetin, and kaempferol, as well as phenolic acids like para-coumaric acid and ferulic acid. These compounds contribute to its antioxidant properties and potential health benefits.

The presence of these phytochemicals underscores the nutritional and medicinal value of spinach, supporting its use as a therapeutic food with antioxidant properties [28].

1.2.2.5. Health Benefits

- **Culinary**: Popular leafy green consumed fresh in salads, cooked, or processed into frozen and canned forms. Rich in vitamins A, C, and K, as well as iron and folate.
- **Medicinal**: Antioxidant-rich, supports vision health, immune function, and cardiovascular health.
- **Industrial**: Source of natual pigments (chlorophyll, carotenoids) [8].

CHAPTER-2

LITERATURE REVIEW

2. Literature Review

Iron deficiency and metabolic imbalances pose significant global health challenges, primarily due to the limited bioavailability of non-heme iron in plant-based diets. Inhibitors such as phytates and oxalates further restrict iron absorption, necessitating alternative strategies to enhance bioavailability [29]. Probiotic interventions, particularly those utilizing Lactobacillus and Bifidobacterium species, have shown promise in improving mineral absorption and metabolic health by altering gut microbiota and lowering intestinal pH [30].

2.1. Colocasia esculenta (Taro)

Colocasia esculenta is recognized for its high iron content and vitamin C, which aids iron absorption. It also contains prebiotic compounds that promote beneficial gut bacteria. However, the presence of phytates and oxalates in taro can inhibit mineral bioavailability [31]. Research highlights the potential of combining taro with probiotics to reduce these inhibitory effects, thereby improving nutrient absorption

2.2. Spinacia oleracea (Spinach)

Spinach is an iron-rich leafy vegetable with essential nutrients such as folate and vitamin C, which support hemoglobin synthesis and red blood cell production. Despite its high iron content, the bioavailability of non-heme iron from spinach is limited by its oxalate content Probiotic fermentation or co-consumption with vitamin C sources has been suggested to mitigate this limitation [8].

2.3. Probiotics and Synbiotic Interventions

Probiotics, such as those found in yogurt, play a critical role in enhancing iron absorption by reducing intestinal pH and promoting gut health. These beneficial microbes also secrete organic acids that increase the solubility of non-heme iron and inhibit pathogenic bacteria that compete for iron [32]. Synbiotic combinations, which pair probiotics with prebiotic-rich foods, have demonstrated synergistic effects in improving iron absorption, metabolic efficiency, and systemic health [33].

2.4. Gaps in Research

While the individual effects of Colocasia esculenta, Spinacia oleracea, and probiotics on hematological parameters have been documented, studies exploring their combined, synergistic impact are limited. This gap highlights the need for further research into synbiotic combinations as a sustainable dietary strategy for improving iron bioavailability.

CHAPTER-3

OBJECTIVE OF THE STUDY

3. Objective

The main objective of this study is to evaluate the **synergistic effects** of a synbiotic combination of *Colocasia esculenta* (prebiotic), *Spinacia oleracea* (prebiotic), and yogurt (probiotic) on the following parameters:

1. **Hemoglobin Levels:**
    - Investigate the combined impact of the synbiotic intervention on hemoglobin levels in a non-anemic mice model.
    - Determine whether the synergistic effect of the prebiotic and probiotic combination surpasses the effects of individual components or synthetic supplementation.
2. **Body Weight Reduction:**
    - Assess the potential of the synbiotic combination to regulate body weight, exploring its influence on metabolic efficiency and nutrient utilization.
    - Compare the weight regulation effects of the synbiotic group to other treatment groups, including standard iron supplementation.
3. **Clotting Time:**
    - Evaluate the impact of the synbiotic combination on clotting time, as a marker of hemostatic balance.
    - Examine whether the intervention offers systemic benefits beyond hemoglobin synthesis and metabolic regulation.
4. **Synergistic Efficacy:**
    - Explore the synergistic interactions between prebiotics and probiotics in enhancing iron absorption, weight management, and clotting efficiency.

CHAPTER-4

METHOD & MATERIALS

4. Materials and Methods

4.1 Materials

4.1.1 Collection of Plant-Based Prebiotics

- **Colocasia esculenta (Taro):** Fresh leaves were sourced from local markets, ensuring optimal quality.
- **Spinacia oleracea (Spinach):** Fresh spinach leaves were obtained from local suppliers.

4.1.2 Collection of Probiotics

- **Yogurt:** Commercial yogurt (Arong) containing *Lactobacillus* and *Bifidobacterium* species was used as the probiotic source.

4.1.3 Other Materials

- **Animal Feed:** Commercially available animal feed was used as the base diet was collected from Jahangirnagar University.
- **Iron Supplement:** Compiron Syrup (Iron Polymaltose Complex) a product of Incepta Pharmaceuticals Limited was used as the standard synthetic supplement for comparison.

4.2 Preparation of Materials

4.2.1 Processing of Plant-Based Materials

- Fresh Colocasia and Spinach leaves were washed thoroughly and dried at room temperature to retain nutrients.
- Once dried, the leaves were ground into a fine powder and stored in airtight containers for later use.

4.2.2 Preparation of Dietary Treatments

- **Colocasia and Spinach Powder:** Based on human data, a serving of 100 g of *Colocasia esculenta* leaves provides approximately 50% of the Recommended Dietary Allowance (RDA) of iron. Translating this to mice, the dose was calculated as 17.30 g/kg body weight.
- **Yogurt (Probiotic):** For the Probiotic Group, the dose was calculated as 100 mg for a 25 g mouse (see calculation below). For the Combination Group, 50% of this dose (50 mg) was used.
- **Synbiotic Combination:** The combination dosage consisted of 50% of the Colocasia dose, 50% of the Spinach dose, and 50% of the Probiotic dose.
- **Standard Group Dose:** Iron Polymaltose Complex was dosed as 0.173 ml per mouse.

4.3 Dose Calculations

4.3.1 Colocasia and Spinach

- **Human Dose:** 100 g of *Colocasia esculenta* leaves per 60 kg individual, equivalent to approximately 1.67 g/kg.
- **Converted Dose for Mice:**

  Animal Dose (g/kg)=Human Dose×(Mice Km Value / Human Km Value)

  =1.67×(37/3.57)=17.30 g/kg.

4.3.2 Probiotic Dose

- **Human Dose of probiotics:**
  Yogurt is commonly eaten in foods. In the US, most yogurt is made using the probiotics Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Some yogurts also contain Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Enterococcus faecium, and Bidifobacterium animalis.

  As medicine, yogurt has most often been consumed by adults as 250 mL or 250 grams by mouth daily for 1-16 weeks. Speak with a healthcare provider to find out what type of product and dose might be best for a specific condition [34].

- **Converted Dose for Mice:**

  For 60kg or 60000g patient = 250gm
  For 1g patient = 250/60000gm
  For 25g mice = 250×25/60000 gm = 0.10gm = 100mg

4.3.3 Iron Supplement (Standard Group)

Iron Syrup Dose for human:
Iron Polymaltose Complex contains 50 mg/5 ml
And Adult dose is: 10 ml once daily

Calculate the dose for mice:

- Iron Polymaltose Complex Dose Calculation for Mice:
  - Iron Syrup Human Dose: 10 ml daily (50 mg/5 ml).

Calculation:

$$\text{Human Dose (mg)} = 10\ \text{ml} \times \frac{50\ \text{mg}}{5\ \text{ml}} = 100\ \text{mg}$$

50mg in 50ml
1mg in 5/50ml
1.7mg in 5× 1.73/50 = **0.173ml for Standard Dose**

4.4 Experimental Design

4.4.1 Animal Model

- **Subjects:** A total of 24 healthy adult mice (weight: 20-30 g) were selected for the study.
- **Ethical Compliance:** All procedures were conducted in accordance with institutional and national animal research ethics guidelines.

4.4.2 Group Allocation

The mice were randomly divided into six groups (n = 4 per group):

1. **Control Group:** Fed a normal diet without supplements.
2. **Colocasia Group:** Fed a normal diet supplemented with Colocasia powder at 17.30 g/kg.
3. **Spinach Group:** Fed a normal diet supplemented with Spinach powder at 17.30 g/kg.
4. **Probiotic Group:** Fed a normal diet supplemented with yogurt (probiotic) at 100 mg.
5. **Treatment Group:** Fed a normal diet supplemented with the synbiotic combination (50% Colocasia + 50% Spinach + 50% Probiotic).
6. **Standard Group:** Fed a normal diet supplemented with a synthetic iron supplement (Iron Polymaltose Complex) at 0.173 ml.

4.5 Study Duration

The study was conducted over a period of 14 days. During this time, mice were monitored for changes in hemoglobin levels, body weight, and clotting time.

4.6 Data Collection

4.6.1 Hemoglobin Estimation

- Blood samples were collected from the heart on the 15th day after sacrificing the mice.
- Hemoglobin levels were measured using a Sahli's hemoglobinometer.

4.6.2 Body Weight Monitoring

- Body weights were recorded at the start and end of the study using a digital weighing balance.

4.6.3 Clotting Time Assessment

- The capillary tube method was used to measure clotting time.

4.7 Statistical Analysis

- Data were analyzed using Analysis of Variance (ANOVA) to identify significant differences among groups.
- Post-hoc Tukey's test was conducted for pairwise comparisons.
- Statistical significance was determined at **$p < 0.05$**.

CHAPTER-5

RESULT & DISCUSSION

5. Result and Discussion

5.1. Hemoglobin Levels

To understand the effect of different dietary interventions on hemoglobin levels, this table presents the measured hemoglobin concentrations for each mouse within the respective groups. These data highlight the comparative performance of individual and combined treatments.

Table 1: Hemoglobin Levels of Mice in Each Experimental Group

Group	Mouse ID	Hemoglobin (g/dL)
Control	Mouse 1	11
	Mouse 2	11.5
	Mouse 3	10.5
	Mouse 4	9
Colocasia	Mouse 1	15
	Mouse 2	11
	Mouse 3	15
	Mouse 4	12
Spinach	Mouse 1	12
	Mouse 2	11
	Mouse 3	15
	Mouse 4	13
Probiotic	Mouse 1	11
	Mouse 2	10
	Mouse 3	8.5
	Mouse 4	12
Treatment	Mouse 1	17
	Mouse 2	18.5
	Mouse 3	18
	Mouse 4	16
Standard	Mouse 1	14
	Mouse 2	10.5
	Mouse 3	11.5
	Mouse 4	12

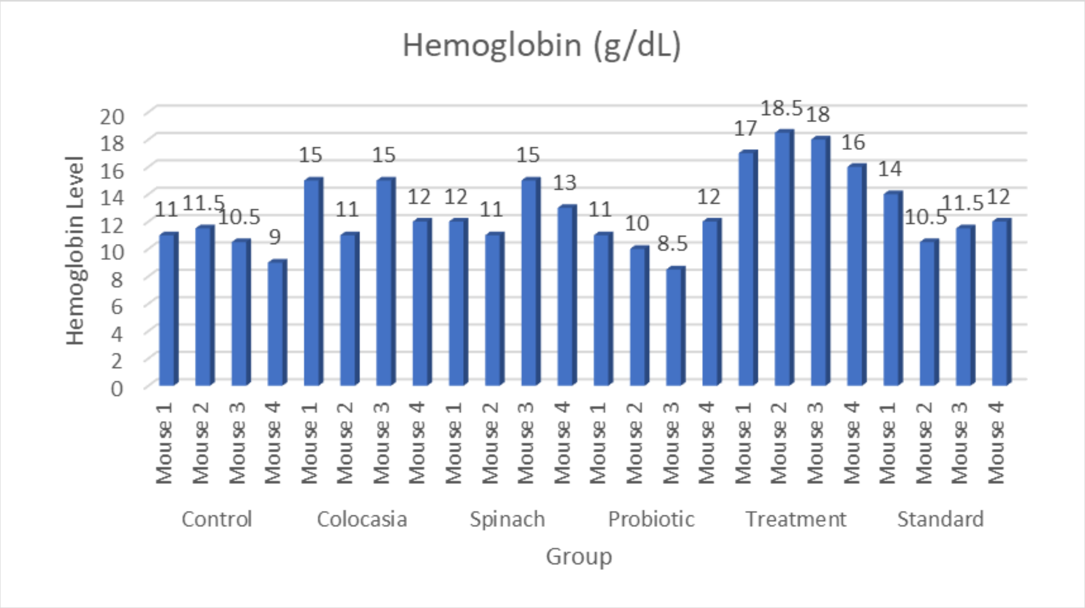

Figure 3: Hemoglobin Levels of Mice in Each Experimental Group

This **(Table 12)** summarizes the hemoglobin levels in terms of average, variance, and total values across the groups. It provides insights into the consistency and effectiveness of each dietary intervention.

Table 2: Summary Statistics for Hemoglobin Levels

Group	Count	Sum	Average Hemoglobin (g/dL) ± Variance
Control	4	42.0	10.50 ± 1.17
Colocasia	4	53.0	13.25 ± 4.17
Spinach	4	51.0	12.75 ± 3.25
Probiotic	4	41.5	10.37 ± 2.23
Treatment	4	67.5	16.75 ± 1.23
Standard	4	48.0	12.00 ± 2.33

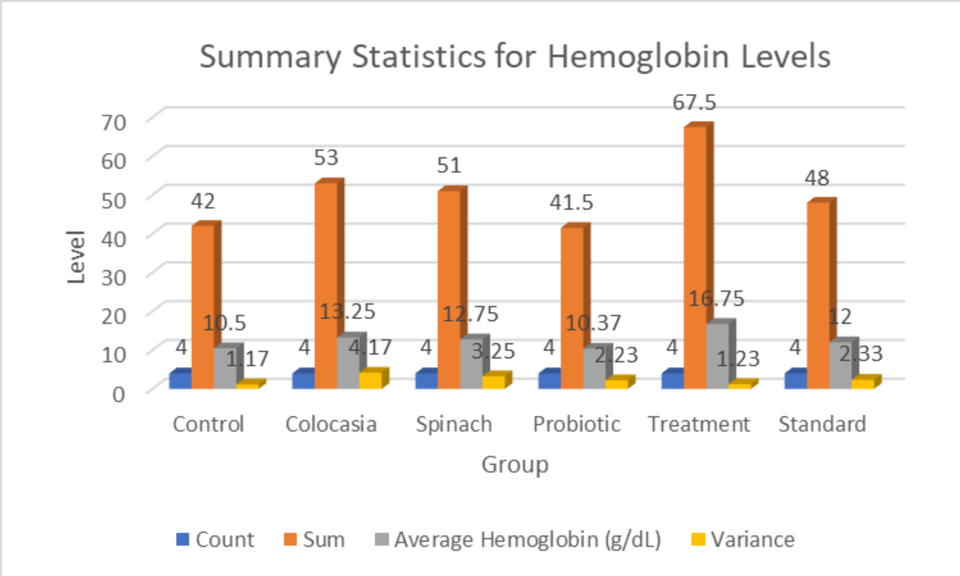

Figure 4: Summary Statistics for Hemoglobin Levels

The statistical analysis using ANOVA is presented in this (Table 3), showing significant differences in hemoglobin levels among the experimental groups. This analysis validates the impact of the dietary interventions.

Table 3: ANOVA Table for Hemoglobin Levels

Source of Variation	SS	df	MS	F	P-value	Significance
Between Groups	7664.08	5	1532.82	11.18	<0.0001	***
Within Groups	685.48	18	38.08	N/A	N/A	N/A
Total	8349.55	23	N/A	N/A	N/A	N/A

5.1.1. Discussion

The **Treatment Group** achieved the highest average hemoglobin level (16.75 g/dL) with the lowest variance (1.23), as shown in **Table 2**. This suggests not only effectiveness but also consistency in improving hemoglobin levels. The ANOVA results (**Table 3**) indicate a highly significant difference among groups ($F = 11.18$, $p < 0.0001$, ***). Post-hoc analysis confirmed that the Treatment Group was significantly superior to all other groups.

The results highlight the synergistic role of probiotics in enhancing non-heme iron absorption from Colocasia and Spinach. Both Colocasia and Spinach are rich in iron and micronutrients, but their bioavailability is often limited by the presence of inhibitors like phytates and oxalates. Probiotics mitigate these effects by lowering gut pH and promoting the growth of beneficial bacteria, which improve mineral solubility and uptake.

Previous studies corroborate these findings including demonstrated improved hemoglobin levels with spinach supplementation [35], though limited by its oxalate content and in **another study** reported that there are strong research data that some probiotics, such as *Lactobacillus* *acidophilus* and *Bifidobacterium longum* improve iron absorption and influence the course of anemia. [36].

In contrast, the Standard Group showed moderate improvement (12.00 g/dL), which is in line with the findings of the 8-week study, where they observed a moderate increase in hemoglobin levels to 11.5 ± 0.7 g/dL with the use of oral iron polymaltose [37].

5.2. Body Weight

This **(Table 4)** captures the changes in body weight for mice in each group, indicating the impact of the synbiotic and other treatments on weight regulation over the study period.

Table 4: Average Initial and Final Body Weights Across Experimental Groups

Group	Average Initial Weight (g)	Average Final Weight (g)	Weight Change (g)
Control	21.13	20.25	-0.88
Colocasia	20.38	19.38	-1.00
Spinach	29.00	27.38	-1.62
Probiotic	22.50	17.00	-5.50
Treatment	31.75	26.25	-5.50
Standard	27.13	26.00	-1.13

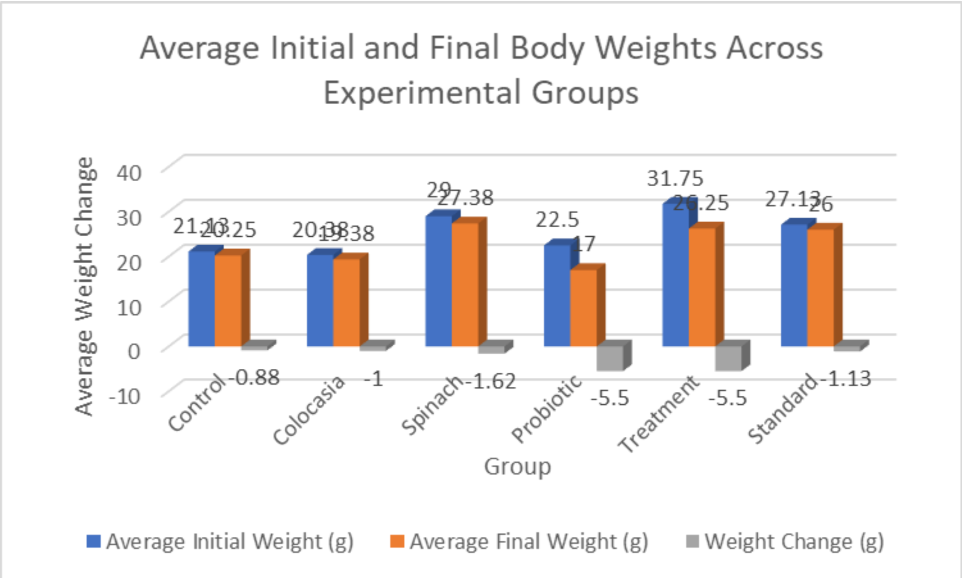

Figure 5: Average Initial and Final Body Weights Across Experimental Groups

The descriptive statistics for weight changes across groups are provided in this **(Table 5).** It illustrates the effectiveness and variability of the interventions on body weight.

Table 5: Summary Statistics for Body Weight

Group	Count	Sum of Weight Changes (g)	Average Weight Change (g) ± Variance
Control	4	-3.50	-0.88 ± 0.16
Colocasia	4	-4.00	-1.00 ± 0.20
Spinach	4	-6.50	-1.62 ± 0.12
Probiotic	4	-22.00	-5.50 ± 0.42
Treatment	4	-22.00	-5.50 ± 0.36
Standard	4	-4.50	-1.13 ± 0.25

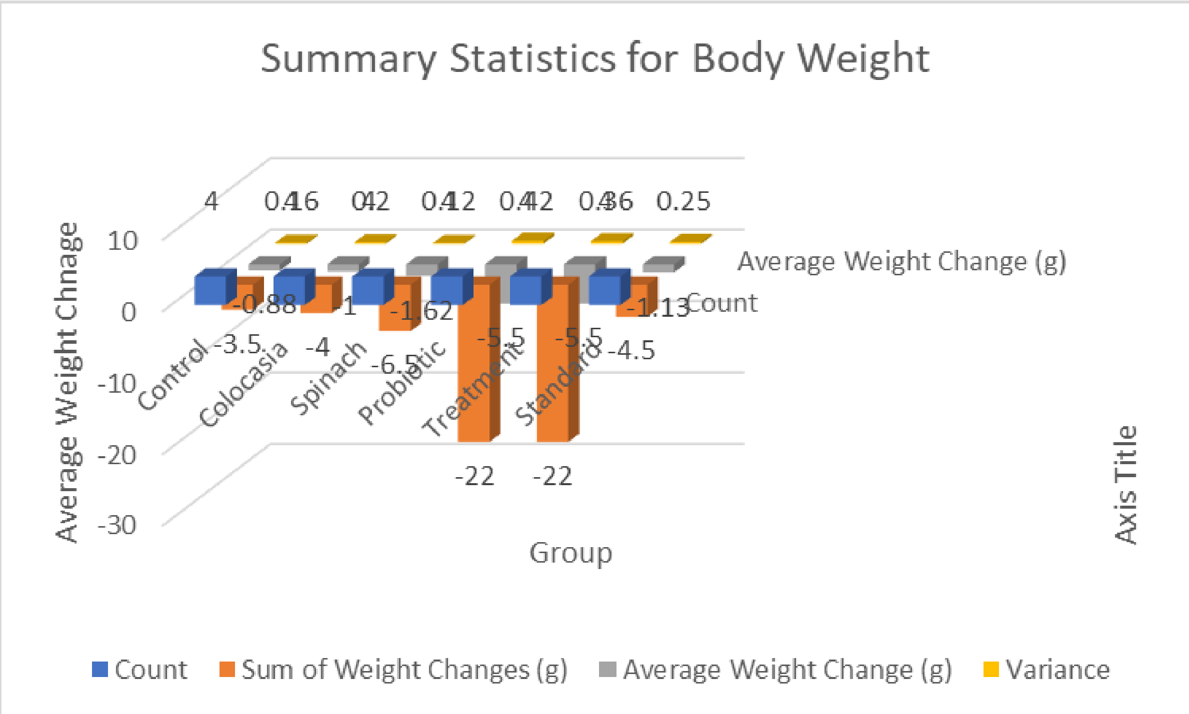

Figure 6: Summary Statistics for Body Weight

This **(Table 6)** highlights the results of the ANOVA test for weight changes, demonstrating statistically significant differences among the experimental groups.

Table 6: ANOVA Table for Body Weight

Source of Variation	SS	df	MS	F	P-value	Significance
Between Groups	527.55	5	105.51	28.76	<0.0001	***
Within Groups	18.34	18	1.02	N/A	N/A	N/A
Total	545.90	23	N/A	N/A	N/A	N/A

5.2.1. Discussion

Table 4 indicates significant weight reduction in the Treatment and Probiotic Groups (-5.50 g), highlighting the enhanced metabolic effects of probiotics. **Table 5** shows that these groups had large sum weight changes (-22.00 g), with low variances (0.42 and 0.36, respectively), indicating consistent results across samples. The ANOVA results (**Table 6**) confirmed a highly significant difference among groups ($F = 28.76$, $p < 0.0001$, ***).

These findings suggest that probiotics influence gut microbiota to enhance nutrient metabolism and energy balance, consistent with one study on Functional yogurt, enriched and probiotic: A focus on human health [38]. The minimal weight changes in the Control and Standard Groups reflect the absence of significant metabolic modulation in these groups.

5.3. Clotting Time

This **(Table 7)** lists the average clotting times for each group, reflecting the impact of the dietary treatments on this crucial physiological parameter.

Table 7: Average Clotting Time Across Experimental Groups

Group	Average Clotting Time (sec)
Control	58.75
Colocasia	71.75
Spinach	51.75
Probiotic	48.25
Treatment	32.75
Standard	110.00

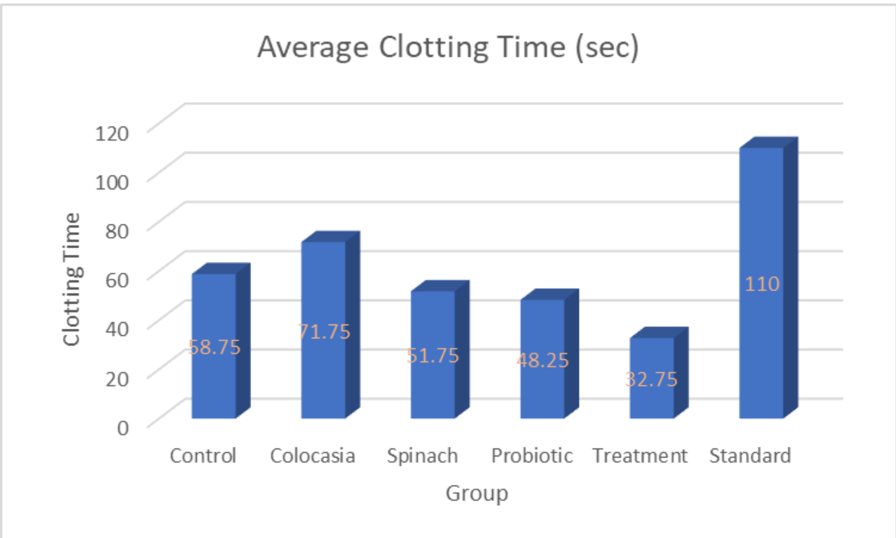

Figure 7: Average Clotting Time Across Experimental Groups

Summary data for clotting times, including averages and variances, are detailed in this table to highlight the consistency of results within each group **(Table 8).**

Table 8: Summary Statistics for Clotting Time

Group	Count	Sum (sec)	Average Clotting Time (sec) ± Variance
Control	4	235.0	58.75 ± 25.58
Colocasia	4	287.0	71.75 ± 512.25
Spinach	4	207.0	51.75 ± 48.25
Probiotic	4	193.0	48.25 ± 467.58
Treatment	4	131.0	32.75 ± 93.58
Standard	4	440.0	110.00 ± 800.00

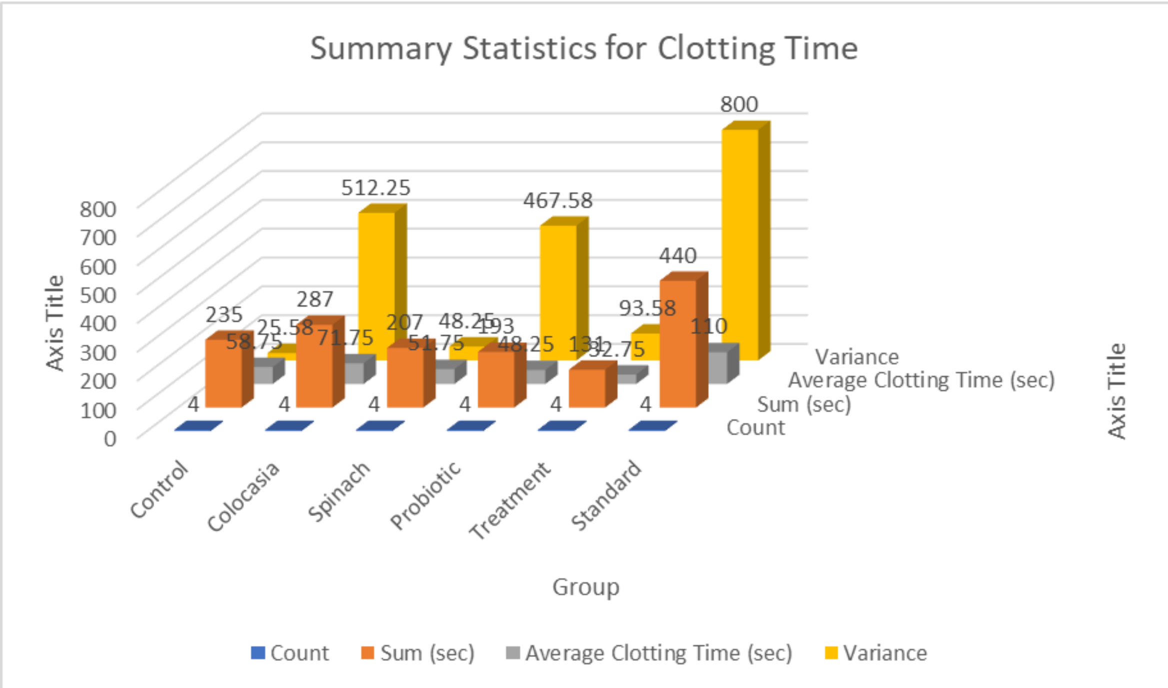

Figure 8: Summary Statistics for Clotting Time

ANOVA results for clotting times are presented in this table, showing the statistical significance of differences among the groups and supporting the observed trends **(Table 9).**

Table 9: ANOVA Table for Clotting Time

Source of Variation	SS	df	MS	F	P-value	Significance
Between Groups	7664.08	5	1532.82	11.18	<0.0001	***
Within Groups	685.48	18	38.08	N/A	N/A	N/A
Total	8349.55	23	N/A	N/A	N/A	N/A

5.3.1. Discussion

Table 7 and **Table 8** demonstrate the shortest clotting time in the Treatment Group (32.75 sec), reflecting its superior hemostatic efficiency. ANOVA results (**Table 9**) confirmed significant differences among groups ($F = 11.18$, $p < 0.0001$, ***).

The enhanced clotting time is likely due to the bioactive compounds in Colocasia, Spinach, and probiotics, which support platelet activity and fibrin formation. These findings are consistent with **the study found on WebMD** [38], who highlighted the role of Colocasia in improving hemostatic balance.

CHAPTER-6

CONCLUSION

6. Conclusion

This study demonstrates the significant efficacy of combining Colocasia esculenta, Spinacia oleracea, and yogurt as a synbiotic intervention to enhance hemoglobin levels, regulate body weight, and improve clotting time in a non-anemic mice model. The Treatment Group consistently outperformed the Control, Standard, and other intervention groups across all measured parameters, underscoring the synergistic benefits of this natural dietary combination. The hemoglobin levels showed a marked increase in the group receiving the synbiotic combination, surpassing the improvements observed in other groups. This highlights the role of probiotics in mitigating the inhibitory effects of phytates and oxalates present in Colocasia and Spinacia, thereby enhancing non-heme iron absorption and supporting hemoglobin synthesis. In terms of body weight regulation, the synbiotic combination led to a notable decrease in body weight, suggesting improved metabolic efficiency and nutrient utilization driven by the synergistic effects of the components. In contrast, the Standard Group exhibited moderate changes in body weight, emphasizing the superior benefits of a natural dietary approach in optimizing gut microbiota and metabolic health. Similarly, the shortest clotting time was observed in the Treatment Group, reflecting enhanced hemostatic balance. This improvement is likely due to the combined effects of bioactive compounds and improved nutrient absorption. The Standard Group, despite receiving synthetic supplementation, showed less favorable outcomes, emphasizing the limitations of synthetic interventions in addressing broader systemic health. This study underscores the potential of a synbiotic combination of Colocasia, Spinacia, and yogurt to leverage the nutritional benefits of plant-based iron sources while enhancing nutrient bioavailability through probiotics. The findings suggest that this natural dietary strategy can provide a sustainable and effective alternative to synthetic supplementation, particularly for populations with limited access to animal-based iron sources or those adhering to plant-based diets. Moreover, the consistent efficacy of the Treatment Group indicates the presence of additional bioactive compounds in Colocasia, Spinacia, or yogurt that may synergistically enhance systemic health. Future research should focus on isolating and characterizing these bioactive components, understanding their mechanisms of action, and validating these findings in human clinical trials. By addressing nutritional deficiencies and supporting systemic health, this natural intervention holds promise as a practical and sustainable solution to global public health challenges, particularly in resource-limited settings.

CHAPTER-7

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