

Article

Anti-Anemic and Anti-Dyspepsia Potential of Yogurt with Carao (*Cassia grandis*) in Rat Model

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Abstract: Iron deficiency anemia is a significant health problem in developing countries and this is rising, particularly in children and pregnant women. Several therapeutic properties have been attributed to *Cassia grandis* (carao), including the treatment against anemia, a laxative effect, and the reduction of bleeding. Yogurt is a vehicle for functional ingredients. As a result, this investigation aims to examine the application of *Cassia grandis* pulp as an anti-anemic and anti-dyspepsia agent in enriched yogurt. Carao pulp powder was added to milk at 0%, 0.5%, 1%, and 3% to produce yogurt. The bioavailability characteristics of iron deficiency anemia were analyzed in albino rats, which were studied for 4 weeks. Other groups of rats were used to set up the dyspepsia model by being fed a high-fat and high-calorie diet. Intestinal propulsion rate, gastric emptying rate, small intestinal contraction, motilin levels, and intestinal muscle tension were analyzed in rats with dyspepsia. Yogurt with 3% carao pulp powder restored ferritin, hemoglobin, total protein and iron at the end of the 4-week feeding period, with significant competition revealed in calcium and zinc absorbance. Furthermore, yogurt with 3% carao pulp powder improved intestinal propulsion rate, gastric emptying rate, small intestinal contraction, motilin levels, and intestinal muscle tension in dyspepsia rats. Carao can be recommended as an anti-anemia supplement in yogurt fortification.

Keywords: anti-anemic supplement; fortified yogurt; carao; albino rats; dyspepsia



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1. Introduction

Cassia grandis, belonging to the *Cesalpinoideae* subfamily, also known as carao, sandalwood, and santal, is a tree native to Central America, the Caribbean, and northern South America [1]. The carao is a tree approximately 30 m high, with compound leaves, pink flowers with an internal yellow spot, and fruit as a woody pod [2]. This tree grows up to 900 m underwater in dry to humid weather. Carao leaves contain anthraquinones, boracol, flavonoids, leuco-anthocyanins, saponins, alkaloids, cardiogenic glycosides, sesquiterpene lactones, tannins, triterpenes, and iron [3]. The carao seed contains flavonoids, polysaccharides, toxic substances, and nutritional inhibitors. Among the latter are phytic acid and tannins [3]. It has been documented that the fresh carao leaf has anti-fungal activity against the *Malassezia furfur* fungus responsible for tinea versicolor or tinea versicolor [1]. Inhibition potential against the mite that causes scabies and against the virus that causes herpes [1] is

also attributed to it. The leaves, flowers, and seeds have been reported to have purgative properties [3]. The flower also has anti-tussive characteristics and antipyretic activity. The bark can assist in treating rheumatism as a healing agent. An antiseptic liquid can be extracted from *cassia grandis* root and applied to disinfected wounds [1]. Alcoholic root extracts are also used for skin diseases. Central American and Caribbean popular culture has used the fruit's pulp for different purposes, such as laxative, anti-tussive, hemorrhage inhibitor, aphrodisiac, menstrual regulator, anti-anemic, and anti-diabetic applications [1].

Yogurt is a food with a high nutritional density that is related to improving the overall diet quality [4]. This is due to its high content of essential ingredients, such as calcium, phosphorus, vitamins of group B, bioactive peptides, fatty acids, essential oils, and lactic acid bacteria (LAB), which complement its high biological value protein and fat content. All of these are part of a food matrix that favors the interaction between them, thus increasing their bioavailability and protecting them from degradation, thanks to their ability to form gels and their viscosity. In this sense, the matrix associates the bioactive peptides with oligosaccharides and fats. α -lactalbumin, β -lactoglobulin, and whey proteins interact with minerals, such as calcium, favoring its absorption [5]. The benefits associated with consuming yogurt seem to be more related to the effect of the matrix than to the impact of isolated nutrients [6]. Traditionally, yogurt consumption's best-known and evidenced effects have been related to gastrointestinal (GI) health benefits [6]. Specifically, its role is highlighted in the treatment of infections, being related to an improvement in the symptoms of acute diarrhea and decreased stool frequency and duration of infection in children [7], as well as in certain diseases of an immuno-inflammatory nature, such as allergies [8] and inflammatory bowel disease (IBD) [9]. In addition, the European Food Safety Authority (EFSA) has recognized the use of the term probiotic for yogurt bacteria, thanks to its β -galactosidase activity, which helps the digestion of lactose and improves intestinal symptoms related to its intolerance [10].

Epidemiological and observational studies have revealed an association between yogurt consumption and metabolic benefits, such as enhanced body weight and adiposity [11] and managing type 2 diabetes [12]. At the same time, their effects on cardiovascular (CV) risk markers and the lipid profile appear neutral [13]. It should be noted that the consumption of other dairy products, such as milk, is not associated with similar effects on metabolism, despite containing a similar nutritional composition, thus suggesting that the milk matrix of the yogurt and its lactic ferments contribute to enhancing the beneficial effects of the nutrients of yogurt [14]. In this sense, yogurt consumption is related to a greater release of beneficial metabolites, such as amino and bile acids, compared with milk consumption [15], suggesting that fermentation is essential to produce metabolites with beneficial properties for human health. In some studies, the effect isolated from the consumption of yogurt can be masked by the inclusion of another type of dairy product in the intervention (milk, cheese, or butter) or by confounding factors that are associated with the consumption of yogurt, such as the practice of a balanced diet or physical activity [14]; however, certain studies show a positive association even after control for these factors [16], thus evidencing a stronger relationship between consumption of yogurt and metabolic benefits.

Paz et al. (2022) [17] and Fuentes et al. (2023) [18] indicated that carao improved the acid and bile tolerance of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Lactobacillus acidophilus* [17,18]. Furthermore, in vitro and in vivo biological properties of yogurt supplemented with *Cassia grandis* showed the potential to treat diabetes mellitus-related disorders [19]. Some studies have indicated that carao yogurt improved glucose levels in diabetic-induced rats and has anti-inflammatory properties in caco2 cells [20]. Nevertheless, there is no research on the effects of yogurt supplemented with carao on anti-anemic and anti-dyspepsia effects. As a result, this investigation aims to examine the application of *Cassia grandis* pulp as an anti-anemic and anti-dyspepsia agent in enriched yogurt. This research aims to study the impact of yogurt fortified with the pulp

of the *Cassia grandis* fruit on improving anti-anemic and anti-dyspepsia characteristics in animal models.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

Between August and September 2022, carao fruit was collected from Catacamas Municipality, Olancho Department, Honduras (Latitude 14.84838° or 14°50'54" north; Longitude −85.89356° or 85°53'37" west). The plant material was manually separated from the whole fruit and was ground using a 501–700 mm knife mill (Retsch SM 100; Retsch GmbH, Haan, Germany). The milled pulp (200 g) was incorporated into 1 L of ethanol solution (97%) at a ratio of 8:2 (*w/v*). The hydroalcoholic solution was filtered through a Whatman # 1 filter paper, and the extract was then concentrated in a rotary evaporator (Rotavapor model 461, Büchi, Flawil, Switzerland). The obtained concentrated solution was lyophilized (Virtis Advantage Pro, SP Scientific, Warminster, PA, USA) and the freeze-dried product kept at room temperature [21].

2.2. Yogurt Preparation

Yogurt was prepared following the method of Aleman et al. (2023) [22] with slight modifications. Carao powder was added to milk (Great Value, Austin, TX, USA) at 3 different concentrations (0.5%, 1%, and 3%) and maintained under continuous mixing for 10 min at 50–55 °C. Whole milk (Great Value, Austin, TX, USA) was standardized to 12% (*w/w*) solids with powdered whole milk (Great Value, Austin, TX, USA). The milk was pasteurized at 85 °C for 30 min, then cooled to 42 °C, to add the starter culture (*S. thermophilus* ST-M5 and *L. bulgaricus* LB-12 (Chr. Hansen, Milwaukee, WI, USA) (1:1 ratio)) directly in a ratio of 3 mg/L of milk. The inoculated milk was incubated for 6 ± 1 h until reaching a pH of 4.5 ± 0.1 , for subsequent cooling at 5 ± 1 °C. Yogurt was freeze-dried in a Free zone (Labconco, Kansas city, MI, USA) freeze-dryer unit for ~72 h [23].

2.3. Animals and Experimental Design

Male albino rats ($n = 36$) weighing 150 to 160 g were obtained from the Faculty of Biology, Universidad Nacional Autonoma de Honduras, Tegucigalpa (Honduras). The Ethics Committee in Food and Nutrition Research endorsed the experimentation protocol at the Honduran Association of Medicine and Nutrition (ASOHMENU) with form # AS-ASHOMENU-0012-2022. Animals were accommodated in cages at room temperature (22 ± 1 °C). For 1 week, rats were fed laboratory chow (protein 18.5%, fiber 11.2%, and fat 2.8%) and water ad libitum to stabilize the rat's metabolic state. After the adaptation week, six groups of seven animals each were established (Figure 1) and randomly distributed. Group 1 consisted of healthy rats (Negative control group), and groups 2 through 6 were induced for anemia by providing for 2 weeks a diet having tannic acid (20 g/kg of body weight) [24]. Group 2 consisted of rats that were induced for anemia but were not fed with yogurt (Positive control group). After rats were induced with anemia, they were fed with free ferrous sulfate, ascorbic acid, and carao extract for 4 weeks, as described by Darwish et al. (2021) [24]. T1 (Group 3), provided yogurt fortified with 125 mgkg^{-1} ascorbic acid and 50 mgkg^{-1} free ferrous sulfate; T2 (Group 4), provided yogurt fortified with 125 mgkg^{-1} ascorbic acid, 50 mgkg^{-1} free ferrous sulfate, and 0.5% of carao pulp extract; T3 (Group 5), provided yogurt fortified with 125 mgkg^{-1} ascorbic acid, 50 mgkg^{-1} free ferrous sulfate, and 1% of carao pulp extract; and T4 (Group 6), provided yogurt fortified with 125 mgkg^{-1} ascorbic acid, 50 mgkg^{-1} free ferrous sulfate, and 3% of carao pulp extract. Once a week for 4 weeks, blood samples were obtained from rats' eyes, and rats' body weights were measured. Under light overnight fasting and diethyl ether anesthesia, rats were sacrificed after the 4 weeks of weight and blood examination. After the rats were sacrificed, blood samples were obtained from the abdominal aorta. Blood samples were centrifuged at 1800 RCF for 20 min at 4 °C, and serum was kept at −20 °C until further analysis. The

heart, liver, kidney, and spleen were aseptically extracted and then set for 24 h in 10% (*v/v*) formalin after necropsy [24].

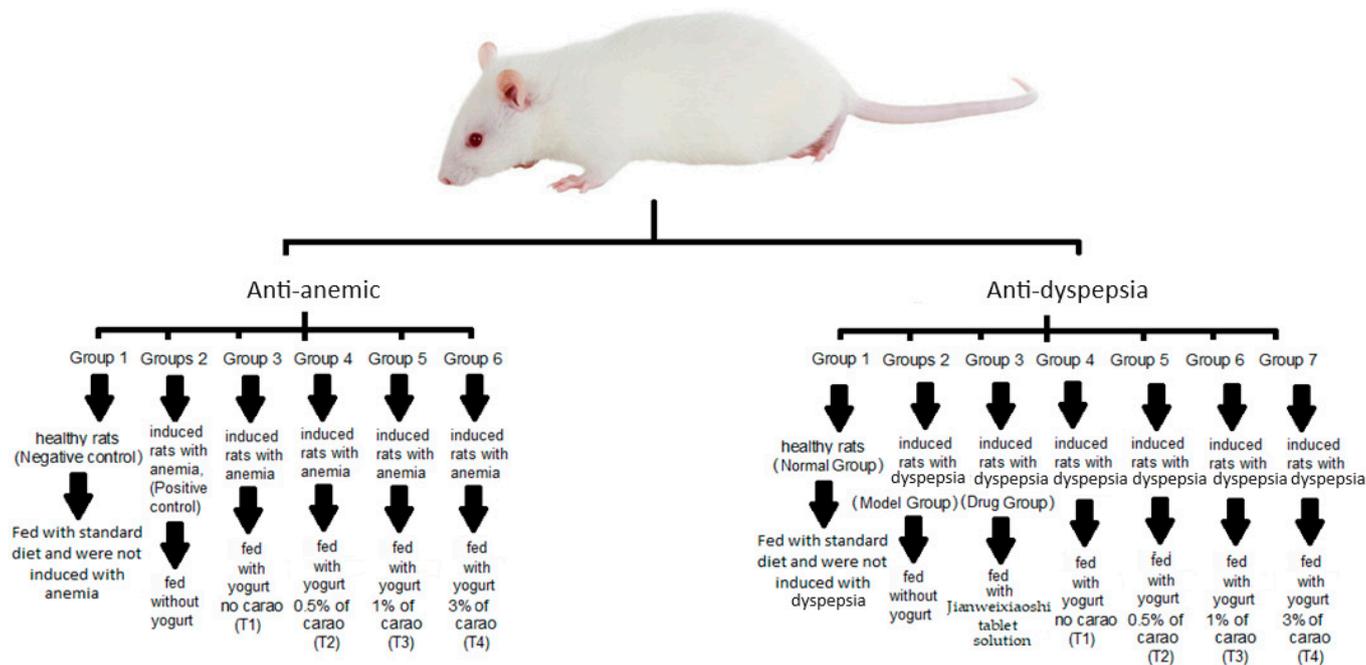


Figure 1. Diagram of induced anemia and dyspepsia in animal models.

Furthermore, other groups of rats were used in a dyspepsia model and fed a high-fat and high-calorie diet. Rats were grouped as follows: drug group (DG), model group, normal group, and yogurt groups (T1, T2, T3, and T4). The rats in the normal group were given a standard diet, and the treatments were fed a high-fat and high-calorie diet, which was administrated with 52% milk from day 1 to day 4 of the experiment. Rats in the yogurt treatments (T1–T4) were provided with 0.3 g of yogurt per rat, and the DG were provided with 0.2 mL Jianweixiaoshi tablet solution (Yancheng, Jiangsu, China) per rat. The normal and model group rats were provided with 0.2 mL of saline solution per rat. T1 was provided with yogurt fortified with 125 mgkg⁻¹ ascorbic acid and 50 mgkg⁻¹ free ferrous sulfate; T2 was provided with yogurt fortified with 125 mgkg⁻¹ ascorbic acid, 50 mgkg⁻¹ free ferrous sulfate, and 0.5% of carao pulp extract; T3 was provided with yogurt fortified with 125 mgkg⁻¹ ascorbic acid, 50 mgkg⁻¹ free ferrous sulfate, and 1% of carao pulp extract; and T4 was provided with yogurt fortified with 125 mgkg⁻¹ ascorbic acid, 50 mgkg⁻¹ free ferrous sulfate, and 3% of carao pulp extract. The rats were sacrificed five days later [25].

2.4. Biochemical Analyses of Anemic Rats

All biochemical analyses were performed similarly to the manner prescribed in the Tietz Clinical Guide [26,27]. The complete blood cell count (RBCs, WBCs, Hb, Platelets, Hct, MCV, MCH, and MCHC) was determined. The automated method used the URIT-3000Vet-Plus equipment for the hematological analysis. First, a drop of blood was positioned on a slide sheet of around 10 microliters; another sheet was placed horizontally on the previous sheet at an angle of 45 degrees and slid horizontally. It was left to dry at room temperature. Then, the staining was performed for 7 min and observed in optical microscopy using immersion oil with a 100× objective to evaluate the total and differential number of leukocytes. The equipment was handled in the following way: once turned on and calibrated, the sample that would have previously passed through the mechanical shaker was positioned, and the examination began so that the machine performed the appropriate reading. The iron forms, such as total iron-binding capacity (TIBC), transferrin saturation (TS), and plasma iron, were examined. Furthermore, albumin, transferrin, ferritin, and the

plasma total protein were examined. Zinc and calcium were also examined to consider their absorbance efficiency with iron. Plasma lipid and glucose profile, as well as kidney and liver function, were studied.

2.5. Analyses of Rats with Functional Dyspepsia

2.5.1. Gastric Emptying Rate and Small Intestine Propulsion Rate

Rats were given 5 g of self-made nutrition sodium carboxymethyl cellulose (semisolid paste), which was dissolved intra-gastrically in 125 mL of water. Whole milk powder (8 g) was then incorporated with activated carbon (2 g), soluble starch (4 g), and glucose (4 g), respectively. The stomach was extracted when mice were sacrificed by cervical dislocation. The weight of the residual substance in the stomach was measured, which was the difference between the net weight of the stomach and the weight of the stomach. The small intestine was also extracted, and the distances from the pylorus to the front of the black semi-solid paste and the pylorus to the ileocecal were estimated. The gastric emptying rate and small intestine propulsion rate were estimated as in Equations (1) and (2) [25].

$$\text{Gastric emptying rate} = 1 - ((\text{Full stomach weight} - \text{Empty stomach weight}) / (\text{semisolid paste weight})) \times 100\% \quad (1)$$

$$\text{Intestinal propulsive rate} = (\text{Charcoal powder advancing distance} / \text{Small intestine full length}) \times 100\%. \quad (2)$$

2.5.2. Small Intestine Muscle Tension and Contraction Frequency

The intestine (2 to 3 cm) was extracted, and both ends of the intestine were tied (one connected to a tension sensor, the other fixed on the specimen hook). The intestine was incubated at 37 °C with 5% CO₂ and 95% O₂. The contraction activity curve was measured with the BL420 bio-functional experimental system. Motilin levels were measured following the kit protocol [25].

2.6. Statistical Analysis

Each experiment was carried out in triplicate or otherwise defined, and the mean and the standard deviations were determined. ANOVA One Way was used followed by Tukey's HSD test using Statistical Analysis Systems SAS (SAS 9.4 Institute Inc., Cary, NC, USA). Significant differences were determined at $\alpha = 0.05$.

3. Results and Discussion

3.1. Impact of Carao Fortified Yogurt Diet on Body and Relative Organ Weights

The relative weights of the spleen, kidney, liver, lungs, and heart of the albino rats as influenced by yogurt fortified with carao are presented in Table 1. The results showed that the relative body weights of the positive control group, the iron-deficiency anemia-induced rats (IDA), were hardly impacted, and showed a significant decrease. In contrast, the yogurt treatments (T1–T4) with fortified carao showed varied significant increases in the relative body weights compared to IDA rats. On the other hand, the plasma biochemical profile of the albino rats as influenced by yogurt fortified with carao is presented in Table 2.

Data regarding body weight and food consumption show great sensitivity in detecting alterations due to low toxicity [28]. The results indicated that yogurt fortified with carao did not cause major changes to the biochemical profile of the albino rats. Several therapeutic properties have been attributed to *Cassia grandis* (carao), including the laxative effect, reduction of bleeding, and treatment against anemia [29].

Table 1. Fortified SFY impact on relative organ weights of treated rats.

Parameters	Negative Control	Positive Control	T1	T2	T3	T4
Total weight gain	14.97 ± 4.49 a	18.8 ± 4.25 a	15.28 ± 3.31 a	20.31 ± 5.97 a	15.55 ± 3.45 a	15.65 ± 3.39 a
Liver (Weight gain)	3.35 ± 1.05 a	3.65 ± 0.95 a	4.05 ± 0.88 a	3.36 ± 0.98 a	3.58 ± 0.81 a	3.25 ± 0.83 a
Kidney (Weight gain)	0.59 ± 0.17 a	0.68 ± 0.17 a	0.79 ± 0.17 a	0.76 ± 0.18 a	0.72 ± 0.16 a	0.70 ± 0.18 a
Spleen (Weight gain)	0.49 ± 0.14 a	0.66 ± 0.16 a	0.83 ± 0.18 a	0.69 ± 0.18 a	0.78 ± 0.18 a	0.62 ± 0.16 a
Heart (Weight gain)	0.43 ± 0.13 a	0.43 ± 0.15 a	0.38 ± 0.08 a	0.36 ± 0.09 a	0.39 ± 0.11 a	0.34 ± 0.09 a
Lungs (Weight gain)	0.73 ± 0.29 a	0.79 ± 0.15 a	0.81 ± 0.17 a	0.74 ± 0.26 a	0.78 ± 0.14 a	0.79 ± 0.22 a

Data represented are means of relative weights (g) (n = 5) ±SD. Means in the same row followed by different letters are significantly different ($p \leq 0.05$). T1, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid; T2, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 3% of carao pulp extract.

Table 2. Fortified SFY impact on plasma biochemical profile of treated rats.

Parameters	Negative Control	Positive Control	T1	T2	T3	T4
Glucose mg dL ⁻¹	193.08 ± 57.94 a	160.55 ± 40.15 a	178.25 ± 39.23 a	175.75 ± 47.45 a	182.85 ± 42.05 a	161.25 ± 41.85 a
T.Ch. mg dL ⁻¹	75.97 ± 25.79 c	79.34 ± 22.32 c	101.01 ± 15.62 b	103.84 ± 22.63 b	95.26 ± 19.61 b	123.31 ± 26.86 a
TG mg dL ⁻¹	173.13 ± 51.94 b	100.22 ± 25.05 a	92.67 ± 15.98 a	101.65 ± 27.44 a	94.52 ± 21.74 a	102.36 ± 31.85 a
HDL mg dL ⁻¹	30.49 ± 9.12 c	42.75 ± 10.64 b	43.72 ± 7.41 b	40.13 ± 10.83 b	38.21 ± 8.74 b	48.45 ± 12.59 a
LDL mg dL ⁻¹	21.65 ± 6.48 b	27.78 ± 6.94 a	26.36 ± 5.79 a	26.83 ± 7.24 a	27.78 ± 6.39 a	32.06 ± 8.33 a
AST mg IU ⁻¹	185.72 ± 55.76 a	203.73 ± 50.94 a	167.91 ± 36.94 a	180.26 ± 48.67 a	161.97 ± 37.25 a	164.58 ± 42.79 a
ALT mg IU ⁻¹	26.36 ± 7.90 a	39.66 ± 9.91 a	29.21 ± 6.42 a	29.96 ± 10.76 a	27.07 ± 6.22 a	28.02 ± 7.27 a
ALP mg IU ⁻¹	274.37 ± 82.29 a	273.12 ± 68.27 a	229.42 ± 50.47 a	249.13 ± 67.26 a	278.82 ± 64.12 a	251.06 ± 65.26 a
T. Bilirubin g dL ⁻¹	0.28 ± 0.05 a	0.22 ± 0.05 a	0.19 ± 0.04 a	0.21 ± 0.05 a	0.27 ± 0.06 a	0.22 ± 0.05 a
Urea mg dL ⁻¹	26.36 ± 7.90 a	35.15 ± 8.78 a	32.3 ± 7.1 a	25.17 ± 6.79 a	25.65 ± 5.88 a	26.36 ± 6.85 a
Creatinine μmol	0.93 ± 0.28 a	0.92 ± 0.23 a	0.91 ± 0.26 a	0.93 ± 0.25 a	0.92 ± 0.21 a	0.93 ± 0.24 a

Means in the same row followed by different letters are significantly different ($p \leq 0.05$). T1, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid; T2, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 3% of carao pulp extract.

3.2. Monitoring Fortification Effects on Complete Blood Count

Figures 2 and 3 show the complete blood count represented in white blood cells (containing lymphocytes, neutrophils, eosinophils, and monocytes), protein hemoglobin (Hb), platelets, and red blood cells (RBCs) of the rat groups over the 4 weeks of the study. The data revealed that the iron-deficiency anemia-induced rats were mirrored in all hematological parameters and lowered levels were indicated in the IDA groups compared to the healthy rats. Yogurt treatments enriched with carao gradually enhanced all hematological parameters in the 4 weeks of the study. This may be due to coating-dependent uptake, dose, and the amount of carao. Additionally, the carao's Fe content may interlink with the results. Targeted functional ingredients are desired for delivery in cells, tissues and organs via local or systemic blood circulation, allowing the ingredients to act directly on the targeted illness sites and, therefore, more effectively generate remedial effects. The literature reports that the *Cassia grandis* has shown probiotic characteristics towards *L. acidophilus* [18]. Medical plants have also shown immuno-stimulant effects in albino rats with aqueous and methanolic extracts [30]. Essential oils have also shown antimicrobial activity in tuberculosis and *S. aureus* [31]. The hypoglycemic effect of carao has been studied carefully and shown to improve the hypoglycemic effect in diabetic rats [32]. In vivo studies have demonstrated the hypoglycemic properties of this plant [33]. Long-term ingestion of iron in large quantities can promote abnormal iron accumulation in the liver. The appearance of hemosiderin, similar to ferritin but with higher iron content and lower solubility, follows the saturation of the apoferritin supply. Hemosiderosis is an iron deposition condition; it occurs in individuals who abnormally consume large amounts of iron or by a genetic defect, resulting in excessive iron absorption [34]. By participating

in iron storage, metabolism, and homeostasis, the liver stores iron in the cytoplasm of hepatocytes in ferritin or hemosiderin granules. The bundle, having among its functions the uptake and destruction of erythrocytes and the subsequent recovery of iron from hemoglobin, stores the iron in ferritin or hemosiderin for future reuse. Like the brain and liver, the bone marrow stores iron in ferritin and hemosiderin [35].

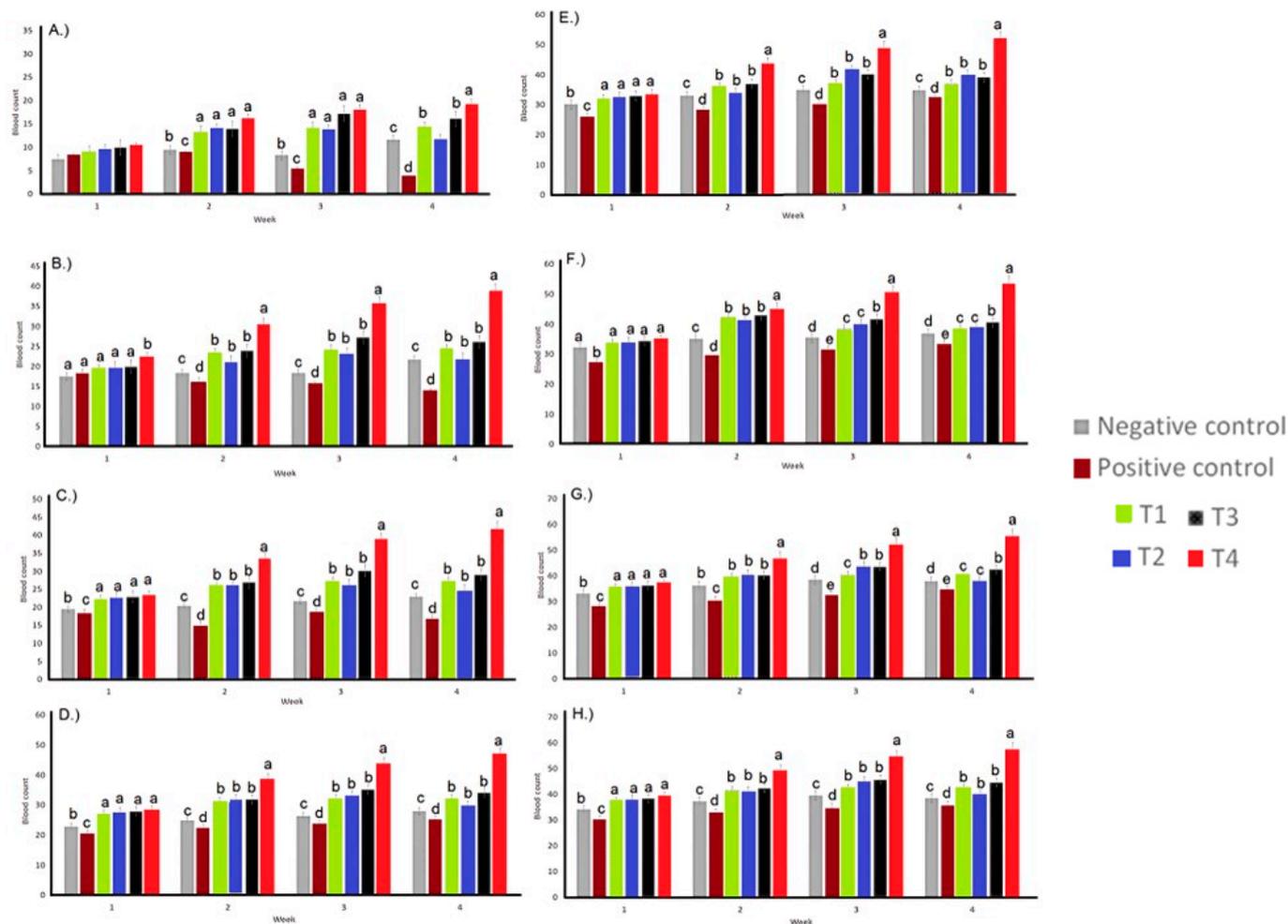


Figure 2. Blood count of RBC (A), HB (B), WBC (C), Neut (D), Lymph/10 (E), Mono (F), Eosino (G), and plt100 (H) as influenced by yogurt fortified by carao. T1, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid; T2, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 3% of carao pulp extract. If there no letters, no statistical difference was detected. Carao fortification is shown to improve the blood count of anemic rats. ^{abcde} Means are statistical different.

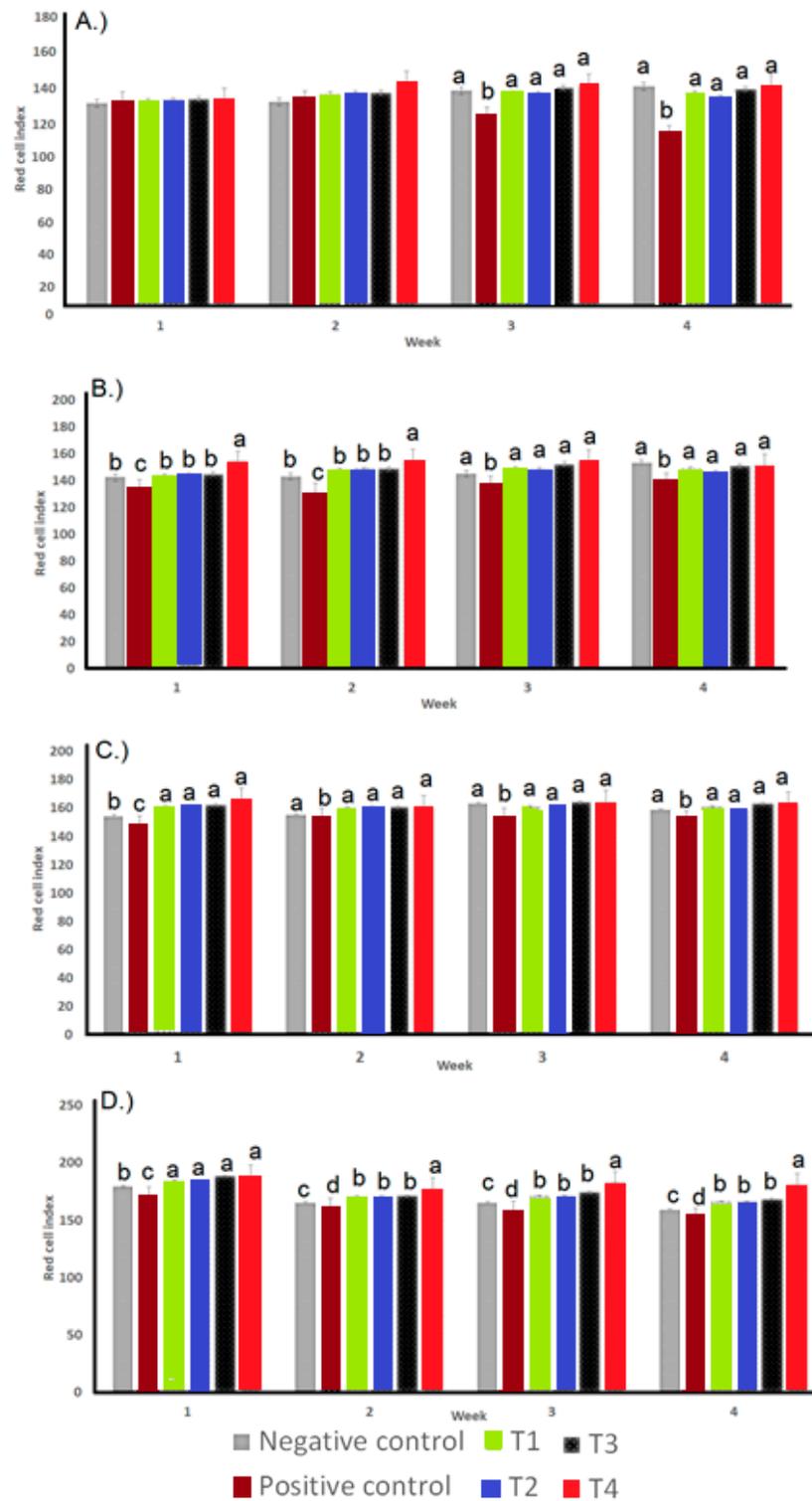


Figure 3. Red cell index of Hct (A), Mkv (B), Mkh (C), and Mkhc (D) as influenced by yogurt fortified by carao. T1, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid; T2, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 3% of carao pulp extract. If there no letters, no statistical difference was detected. ^{abcd} Means are statistical different.

3.3. Fortification Effects on Serum Profile of Rat Groups

Figures 4 and 5 illustrate the effect of enrichment on the rat groups' serum protein and mineral profiles. Serum Fe parameters were negatively impacted, indicating marked reductions in the iron-deficiency anemia-induced rats (Figure 4). Yogurt supplemented with carao restored serum Fe levels with varied responses. The zinc and calcium levels were disproportionately associated with the carao enrichment levels (Figure 6). The Fe–Zn and Fe–Ca competition and interactions in absorbance have been previously studied; over a degree of “physiological” calcium intake, Fe absorption was disproportionately associated to calcium content in food [36]. Conversely, the serum protein profile illustrates different trends (Figure 5). The iron-deficiency anemia-induced rats presented the most inferior ferritin levels. Ferritin levels were significantly improved when treated with yogurt supplemented with carao. A similar trend was reported for transferrin and total protein.

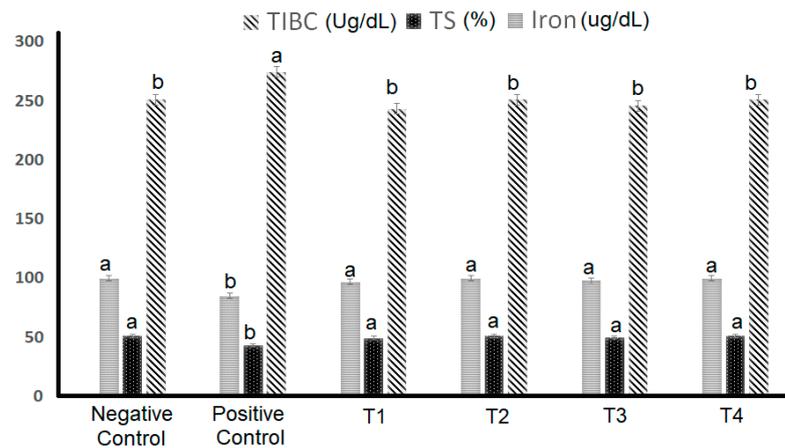


Figure 4. Iron parameters of rat groups as influenced by yogurt fortified by carao. T1, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid; T2, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 3% of carao pulp extract. ^{ab} Means are statistical different.

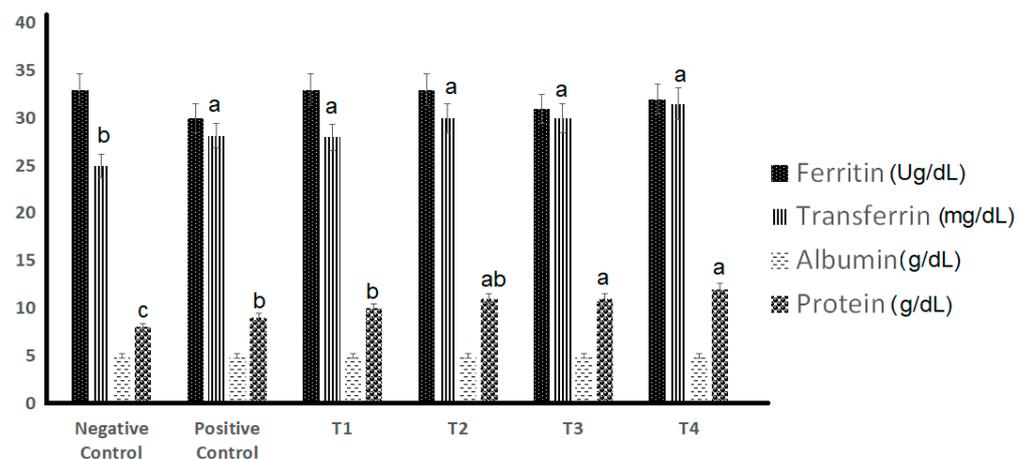


Figure 5. Protein parameters of rat groups as influenced by yogurt fortified by carao. T1, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid; T2, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 3% of carao pulp extract. If there no letters, no statistical difference was detected. ^{abc} Means are statistical different.

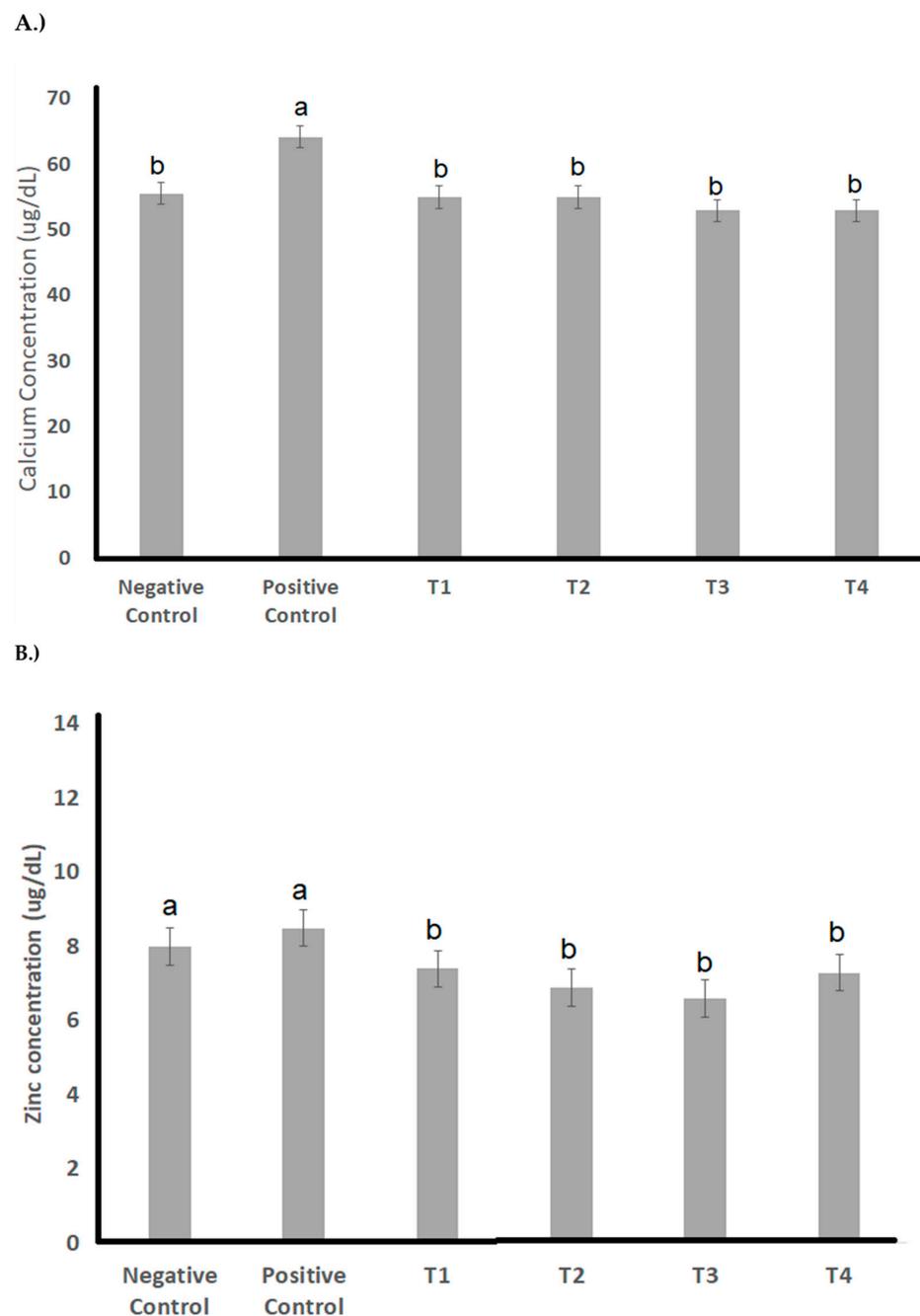


Figure 6. Calcium concentration (A) and Zinc concentration (B) of rat groups as influenced by yogurt fortified by carao. T1, fed SFY fortified with 50 mgkg^{-1} free ferrous sulfate + 125 mgkg^{-1} ascorbic acid; T2, fed SFY fortified with 50 mgkg^{-1} free ferrous sulfate + 125 mgkg^{-1} ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg^{-1} free ferrous sulfate + 125 mgkg^{-1} ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg^{-1} free ferrous sulfate + 125 mgkg^{-1} ascorbic acid + 3% of carao pulp extract. ^{ab} Means are statistical different.

However, the albumin levels were not significantly affected. If we analyze the *ex vivo* effect of the aqueous carao extract on smooth muscle, it can be concluded that it generally produces an increase in contraction. The specificity of smooth muscle is governed by the fact that different smooth muscles have different complements of agonist receptors. In many cases, a variety of different agonists can act on a single cell type, while some transmitters or hormones antagonize smooth muscle contraction and relaxation [37].

Abramson et al. (1988) [38] report that some anthraquinones induce the release of calcium from the sarcoplasmic reticulum in skeletal muscle. These authors explain that this effect is a direct consequence of the interaction between anthraquinones and the ryanodine receptor located in the membrane of the sarcoplasmic reticulum; therefore, it could be assumed that the active component contained in the aqueous carao extract does not bind to a receptor on the plasma membrane, if not to a cytosolic receptor. The aqueous extract of the pulp of the fruit of *Cassia grandis*, in addition to containing anthracene glycosides, contains cinnamic acid. The presence of said component in the aqueous carao extract had previously been identified by Hernández (1978) [39].

Cinnamic acid is a phenolic monomer found in a wide variety of plants [39]. After oral administration, it is excreted unchanged in the urine [40,41]. It has been reported that cinnamic acid is absorbed in the jejunum through different transport channels. Therefore, it is important to emphasize that plant extracts present several components in different proportions and that the compound found in the greatest quantity does not always produce the biological effect studied.

3.4. Fortification Effects on Gastric Emptying Rate, Intestinal Propulsion Rate, Intestinal Muscle Tension, and Small Intestinal Contraction Frequency

The small intestinal contraction frequency, intestinal propulsion rate, gastric emptying rate, and intestinal muscle tension in the model group decreased when compared with the healthy rats (Table 3). On the other hand, the gastric emptying rate, intestinal propulsion rate, intestinal muscle tension, and small intestinal contraction frequency in the yogurt treatments with carao and drug group increased when compared with the model group (Table 3).

Table 3. Effect of yogurt on gastric emptying rate, intestinal propulsion rate, intestinal muscle tension, small intestinal contraction, and motilin level frequency in dyspepsia rats.

Parameters	Normal Group	Model Group	Drug Group	T1	T2	T3	T4
Gastric emptying rate	46.12 ± 1.8 d	34.60 ± 1.4 e	62.20 ± 8.3 a	54.60 ± 3.1 c	55.76 ± 3.1 c	56.65 ± 4.13 bc	58.43 ± 1.45 b
Intestinal propulsion rate	48.45 ± 3.1 a	33.00 ± 8.3 e	41.00 ± 1.4 b	35.23 ± 3.2 d	35.76 ± 1.3 d	36.33 ± 2.45 d	38.80 ± 1.6 c
Intestinal muscle tension	0.14 ± 0.02 a	0.08 ± 0.01 d	0.16 ± 0.04 a	0.11 ± 0.01 c	0.12 ± 0.01 c	0.12 ± 0.01 bc	0.13 ± 0.01 b
Small intestinal contraction frequency	6.25 ± 0.60 d	6.00 ± 0.71 e	7.56 ± 1.58 a	7.46 ± 1.59 c	7.48 ± 1.45 cb	7.48 ± 1.27 cb	7.50 ± 1.33 b
Motilin level	2023.77 ± 365.64 c	1101.77 ± 275.75 b	1221.19 ± 392.92 a	1105.56 ± 223.57 b	1045.56 ± 222.90 b	1007.56 ± 257.88 b	1001.56 ± 228.74 b

Means in the same row followed by different letters are significantly different ($p \leq 0.05$). T1, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid; T2, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 0.5% of carao pulp extract; T3, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 1% of carao pulp extract; and T4, fed SFY fortified with 50 mgkg⁻¹ free ferrous sulfate + 125 mgkg⁻¹ ascorbic acid + 3% of carao pulp extract.

Gastric emptying is a state that induces a delay in emptying food from the stomach into the duodenum, a complete embodiment of gastric motility. Slowed gastric emptying is the primary embodiment of gastrointestinal illnesses [42]. Rats were provided with high-calorie, high-protein foods to induce dyspepsia. The excessive consumption of high-calorie, high-protein foods inhibits normal function by reducing the gastrointestinal motility and transportation of the gastrointestinal tract.

The gastric emptying rate of mice with yogurt treatments and positive control were higher than the model group, suggesting that yogurt with carao has a positive impact and can stimulate gastric emptying. The small intestinal propulsive rate is associated with an increase in the duration of gastrointestinal mechanical movement and is one of the classical indexes of gastrointestinal mechanics. The gastric emptying hold and peristalsis delay are the primary pathophysiological causes of gastrointestinal motility illnesses [43]. The small intestine propulsion of mice with yogurt treatments and positive control were higher than the model group, suggesting that yogurt with carao has a positive impact and can improve

gastrointestinal peristalsis function. ‘Motility disorders’ is an umbrella term that includes many illnesses that can impact the additional components of the gastrointestinal tract, and the gastrointestinal movement regulates peristalsis and segmentation [44].

The small intestine muscle tension of mice with yogurt treatments and positive control were higher than the model group, suggesting that yogurt with carao has a positive impact and can improve dyspepsia rat’s small intestine muscle tension. This observed effect could have occurred due to the action of the digestive enzymes of the intestine or the intestinal flora, which released or formed some compound that produced relaxation. In the literature, it has yet to be reported that the same extract presents two contrasting effects. However, Akomolafe et al. (2004) [45] reported that, in rats, the aqueous extract of *Cassia podocarp* produces colon contraction, while the methanolic extract produces relaxation of the colon and ileum.

In addition, the aqueous extract of *Cassia acutifolia* relaxes the colon and ileum, while the methanolic extract produces colon contraction. According to Kuo et al. (2000) [46], anthraquinones with a polar group, such as an amino, aldehyde, or carboxylic acid, significantly reduce intestinal motility. From the physiological point of view of smooth muscle contraction and relaxation, the relaxing effect observed with the aqueous extract of carao could also be explained through the negative feedback mechanism mediated by K^+ channels activated by Ca^{2+} , where the increase of calcium in the cytosolic space, determined by the previous increase in contraction, activates K^+ channels that hyper-polarize the cell and promote relaxation [47–49].

4. Conclusions

The application of *Cassia grandis* pulp as an anti-anemic and anti-dyspepsia agent enriched in yogurt was investigated. Yogurt with 3% carao pulp powder improved hemoglobin, iron, ferritin, and total protein levels in albino rats at the 4-week feeding period, with considerable zinc and calcium absorbance. Furthermore, yogurt with 3% carao pulp powder improved gastric emptying rate, intestinal propulsion rate, intestinal muscle tension, small intestinal contraction, and motilin levels in dyspepsia rats. In summary, carao can be recommended as an anti-anemia supplement in yogurt fortification.

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