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ORIGINAL PAPER

Is the impact of copper nanoparticles on the immune system of rats dependent on the diverse physiological functions of dietary fibre?

A. Marzec[1], B. Fotschki[2], E. Cholewińska[1], W. Dworzański[3,4], J. Juśkiewicz[2] and K. Ognik[1,*]

[1] University of Life Sciences in Lublin, Faculty of Animal Sciences and Bioeconomy, Department of Biochemistry and Toxicology, Akademicka 13, 20-950 Lublin, Poland
[2] Polish Academy of Sciences, InLife Institute of Animal Reproduction and Food Research, Trylińskiego 18, 10-683 Olsztyn, Poland
[3] Casimir Pulaski Radom University, Faculty of Medical and Health Sciences, Department of Non-Procedural Clinical Sciences, Bolesława Chrobrego 27, 26-600 Radom, Poland
[4] Casimir Pulaski Radom University, Faculty of Medical and Health Sciences, Clinical Department of Cardiology with Electrotherapy Laboratory, Juliana Aleksandrowicza 5, 26-617 Radom, Poland

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ABSTRACT. A six-week feeding trial was conducted to evaluate the effects of dietary supplementation with copper nanoparticles (CuNPs) administered at 6.5 or 13.0 mg Cu/kg diet, in combination with different types of dietary fibre, on haematological and immune parameters in rats. Ten experimental groups were included. Two control groups received cellulose-based diets containing copper(II) carbonate($CuCO_3$) at 6.5 or 13.0 mg Cu/kg diet. In the experimental groups, $CuCO_3$ was replaced with CuNPs at the corresponding concentrations, and diets were supplemented with various fibre types: cellulose, pectin, inulin, or psyllium. At the end of the experimental period, blood samples were collected for the assessment of haematological and immune indices. Irrespective of Cu form or dose, diets containing pectin or inulin lowered the counts of white blood cells (WBC) and lymphocytes (LYM), while inulin or psyllium reduced interleukin 6 (IL-6) concentrations. In contrast, CuNPs administered at 6.5 mg Cu/kg diet in combination with psyllium increased WBC and LYM counts. The inclusion of inulin or psyllium in diets containing the lower CuNPs dose decreased immunoglobulin M (IgM), IL-6, and tumour necrosis factor α (TNF-α) levels. Replacing $CuCO_3$ with CuNPs at 6.5 mg Cu/kg in diets containing cellulose, inulin, or psyllium lowered immunoglobulin A (IgA) levels, while cellulose additionally increased C-reactive protein (CRP) concentrations. A diet containing double dose of CuNPs (13.0 mg Cu/kg) with psyllium reduced mean corpuscular haemoglobin (MCH) and increased red cell distribution width (RDWc) and mean platelet volume (MPV). Moreover, high-dose CuNPs diets containing pectin, inulin, or psyllium lowered IgA, IgM, interleukin 2 (IL-2), and TNF-α concentrations, and pectin additionally reduced immunoglobulin G (IgG) levels. In summary, replacing standard $CuCO_3$ with CuNPs, even at 6.5 mg Cu/kg diet, induced inflammation and impaired immune function in rats. However, supplementation with pectin or inulin alleviated the adverse immune and inflammatory effects caused by Cu nanoparticles.

* Corresponding author:
e-mail: katarzyna.ognik@up.edu.pl

Introduction

Copper (Cu) is an essential dietary element for both humans and animals. It performs multiple physiological functions, including acting as a cofactor for numerous metabolic enzymes, participating in respiratory chain energy production, neutralising free radicals, as well as maintaining proper connective tissue structure and nerve conduction (Angelova et al., 2011; Opazo et al., 2014; Bost et al., 2016; Tishchenko et al., 2016). The recommended dietary allowance (RDA) for Cu in human adults is 900 μg/day, and the tolerable upper intake level (UL) is 10000 μg/day (10 mg/day), established based on liver damage as the critical adverse effect (NIH, 2025). Substantial evidence indicates that Cu also plays an important regulatory role in immune homeostasis and inflammatory responses (Stafford et al., 2013; Cheng et al., 2022; Liu et al., 2022; Lan et al., 2024; Li et al., 2024; Lu et al., 2024). Recent studies have demonstrated that Cu directly binds to alpha kinase 1 (ALPK1), a cytosolic pattern recognition receptor, leading to its activation. This interaction enhances innate immune signalling via the nuclear factor kappa B (NF-κB) pathway and stimulates the production of pro-inflammatory cytokines, including interleukin 8 (IL-8), interleukin 1β (IL-1β), and tumour necrosis factor α (TNF-α) (Lu et al., 2024).

Cu deficiency has been shown to seriously impair both innate and adaptive immunity. It has been associated with reduced neutrophil counts, excessive suppression of reactive oxygen species (ROS) production, unfavourable alterations in T lymphocyte populations, in particular, a decrease in CD4+ helper T cells, and impaired IL-2 synthesis (Cheng et al., 2022; Lan et al., 2024). Cu is also required to maintain macrophage antimicrobial activity. Pro-inflammatory signals promote Cu accumulation in phagolysosomes, where it supports ROS generation necessary for pathogen elimination (Stafford et al., 2013). Furthermore, Cu acts as a cofactor of superoxide dismutase, a key antioxidant enzyme; its deficiency increases oxidative stress and activates inflammatory pathways (Li et al., 2024). During infection, elevated serum Cu levels have also been observed, accompanied by increased ceruloplasmin activity, which represents an important component of the acute-phase immune response (Li et al., 2024). Mice lacking the ceruloplasmin gene were found to be highly susceptible to bacterial infections and displayed a diminished cytokine response (Liu et al., 2022). Collectively, these findings demonstrate that an optimal Cu status is indispensable for effective immune responses, whereas deficiency leads to significant immunosuppression and increased susceptibility to infections.

Traditionally, inorganic Cu salts such as copper(II) carbonate ($CuCO_3$) have been used as dietary supplements; however, their bioavailability is relatively low (Ognik et al., 2016). In recent years, Cu nanoparticles (CuNPs) have been proposed as a more efficient source of this element. They are more readily absorbed from the gastrointestinal tract compared to $CuCO_3$ and have been shown to positively stimulate immune system responses (Cholewińska et al., 2018). Moreover, CuNPs more effectively inhibit protein oxidation and nitration, thereby preventing protein degradation, limiting DNA methylation (Ognik et al., 2019), and improving the antioxidant status of the liver and brain (Ognik et al., 2020). Despite these potential advantages, the use of CuNPs as dietary supplements is not without risks, particularly in relation to immune and inflammatory processes. Their higher bioavailability may be associated with stronger cytotoxic effects compared to traditional Cu salts. Tulinska et al. (2022) demonstrated that CuO nanoparticles significantly increased T lymphocyte proliferation and the production of both Th1 (IFN-γ, IL-12p70) and Th2 cytokines (IL-4, IL-5), while concurrently suppressing granulocyte phagocytic activity and reducing glutathione levels in mice. In addition, the 'Trojan horse' mechanism attributed to Cu nanoparticles, involving endocytic uptake and subsequent dissolution in lysosomes, leads to intracellular Cu overload, reaching millimolar concentrations in both the cytoplasm and the nucleus. This may induce extensive alterations in the expression of genes related to oxidative stress, DNA damage responses, and inflammatory pathways (Strauch et al., 2017). Our previous study (Cholewińska et al., 2018) has demonstrated that, owing to their antimicrobial properties, Cu nanoparticles markedly reduce the enzymatic activity of beneficial gut bacteria and decrease the production of short-chain fatty acids, which may impair the function of gut-associated lymphoid tissue. Considering all these factors, further research on the regulation of Cu absorption, particularly in nanoparticle form, appears to be essential.

Dietary fibre represents another crucial dietary component, defined as various plant-derived substances that are resistant to digestion by enzymes of human and monogastric animal digestive tracts. Many studies in rats have demonstrated that fibre supplementation, particularly in high-fat diets, improves lipid profiles and limits body weight gain, resulting in values comparable to those observed

in animals fed low-fat diets (Artiss et al., 2006; Lecumberri et al., 2007). Beneficial effects of dietary fibre on gut microbiota composition and maintenance of the intestinal barrier have also been reported (Lee et al., 2015). Different types of dietary fibre significantly influence the pH in the lumen of the small and large intestines, e.g., by increasing water-binding capacity or digesta viscosity. These changes improve gut acidity and may consequently influence Cu absorption (Aggett and Fairweather-Tait, 1998). Fermentation of soluble dietary fibre by the gut microbiota leads to the production of short-chain fatty acids (SCFAs), which lower the pH in the colon and, to a lesser extent, in the ileum. Higher acidity increases Cu solubility and ionisation, thereby facilitating its intestinal absorption. Conversely, a more alkaline environment, frequently associated with low intake of fermentable fibre, may cause Cu to precipitate in the form of insoluble hydroxides, reducing its bioavailability (Wu et al., 2021). Certain types of dietary fibre, particularly those rich in phytates and polyphenols, may also chelate Cu ions and form insoluble complexes, directly inhibiting Cu absorption in the intestinal lumen. In addition, viscous and gelling fibres, such as pectin, increase digesta viscosity and may physically hinder the interaction of Cu ions with transport proteins located on the surface of enterocytes. The increased water-holding capacity of dietary fibre slows gastric emptying and intestinal passage, potentially prolonging or modifying the period available for Cu absorption (Baye et al., 2017; Cholewińska et al., 2023). Studies have also indicated that manipulating dietary fibre content can also indirectly influence Cu bioavailability by altering the intestinal availability of mineral antagonists (Baye et al., 2017). Dietary fibre can alter the bioavailability of minerals such as zinc, calcium, and iron, which share identical or overlapping transport pathways with Cu and may compete for binding sites on mucosal transporters. Moreover, the form of fibre, its fermentability, and degree of polymerisation have been shown to determine both the magnitude and the direction of its effect on the absorption of Cu and other trace elements (Wapnir, 1998; Baye et al., 2017). Thus, the combination of CuNPs with different types of dietary fibre represents a promising direction in research on immune system modulation, as it utilises synergistic potential to limit toxicity while supporting beneficial immune responses. CuNPs, owing to their higher bioavailability compared to conventional Cu salts, suppress the enzymatic activity of the gut microbiota and reduce the production of SCFAs. However, our previous studies have demonstrated that these effects are strongly reduced by functional dietary fibres such as inulin, pectin, and psyllium (Juśkiewicz et al., 2024). As in our previous studies, four different dietary fibre compounds were evaluated, representing distinct functional classes, i.e., cellulose, pectin, inulin, and psyllium. Cellulose was used as a control inert fibre. Turnlund et al. (1985) demonstrated that dietary α-cellulose does not reduce Cu absorption, which provided the rationale for selecting this fibre type as a control. Pectin represents a viscous and gelling fibre, inulin is a prebiotic stimulating the gut microbiota, while psyllium is a bulking fibre that increases stool mass. Our earlier findings indicated that these different fibre types interact differently with CuNPs, which may significantly affect immune system function (Cholewińska et al., 2023; Juśkiewicz et al., 2024; Marzec et al., 2025). Among the fibres tested, inulin was the most effective in restoring butyrate and propionate production even in the presence of CuNPs, which may support the formation of an anti-inflammatory microenvironment through increased SCFA-mediated immunomodulation. In contrast, pectin exhibited the strongest capacity to rapidly increase bacterial enzyme activity and sustain beneficial microbial metabolic functions. Meanwhile, psyllium reduced ammonia formation and the production of putrefactive SCFAs, thereby minimising intestinal inflammatory load (Juśkiewicz et al., 2024). The protective effects of dietary fibre also involve reinforcement of intestinal barrier integrity through increased expression of tight junction proteins and reduced oxidative stress (Cholewińska et al., 2023). In addition, dietary fibre supplementation modifies the kinetics of CuNPs absorption, facilitating controlled release and reducing systemic toxicity, while simultaneously preserving the selective antimicrobial activity of nanoparticles. This activity preferentially targets pathogenic bacteria while protecting lactic acid bacteria (*Lactobacillus*) and other commensals (Lamas et al., 2020; Juśkiewicz et al., 2024). It was therefore hypothesised that dietary supplementation with different fibre types, in combination with CuNPs, would create a unique immunomodulatory environment, enabling the safe and targeted action of CuNPs on the immune system and inflammatory processes. Current evidence regarding the precise *in vivo* immunomodulatory effects of specific fibre types, administered together with Cu nanoparticles, remains limited, particularly in relation to their interaction with the systemic immune response. Previous studies have focused primarily on the separate effects of CuNPs or dietary fibre, or have not comprehensively addressed their synergistic or antagonistic interactions

affecting immune function and metabolic outcomes during co-administration. Expanding this body of evidence is essential for the development of dietary strategies that safely utilise the enhanced bioactivity of Cu nanoparticles while minimising potential health risks.

Based on experimental evidence from animal models, dietary fibres protect against CuNPs-induced oxidative damage and inflammation, primarily by improving intestinal barrier function. However, indirect mechanisms involving modulation of the gut microbiota or systemic immune responses cannot be excluded. Cholewińska et al. (2023) observed that bioactive pectin- and fructan-type fibres strengthened the intestinal barrier, most likely by increasing mucus viscosity, intestinal content mass, and the expression of protective barrier proteins (e.g., ZO-1). These fibres also reduced CuNPs absorption, presumably by binding Cu ions in the gut lumen and limiting their uptake. The gut microbiota has been identified as a key regulator of intestinal immunity under exposure to nanomaterials, and ingested metal nanoparticles markedly alter the enzymatic and metabolic activity of large-intestinal microorganisms (Tang et al., 2021; Cholewińska et al., 2023; Juśkiewicz et al., 2024; Marzec et al., 2025). Therefore, it was hypothesised that dietary CuNPs supplementation, combined with a neutral control substance (cellulose), a prebiotic (inulin), a viscous fibre (pectin), or a bulking fibre (psyllium), would influence physiological responses and thereby regulate the immunological effects of CuNPs. The objective of the study was to determine whether the inclusion of different types of dietary fibre, i.e., inulin, pectin, or psyllium, in diets containing the lower or a twofold higher dose of CuNPs (6.5 or 13.0 mg Cu/kg) would improve immune function.

Material and methods

The present study is a part of a broader research initiative aimed at investigating the impact of dietary CuNPs in combination with different types of dietary fibre (cellulose, inulin, pectin, and psyllium) on various aspects of the biological response in rats. Accordingly, the experimental design and methodological procedures have been described in detail in studies previously published by the authors (Cholewińska et al., 2023; Majewski et al., 2023; Juśkiewicz et al., 2024; Marzec et al., 2024; 2025).

Copper nanoparticles and dietary fibre

Cu nanoparticles (Cu^0) were purchased from Sky Spring Nanomaterials, Inc. (Houston, TX, USA). The material was supplied by the manufacturer as a nanopowder with 99.9% purity, a nominal particle size of 40–60 nm, and a predominantly spherical morphology. The reported bulk density was 0.19 g/cm³, while the true density was 8.9 g/cm³. The experiment used the same metallic CuNPs as in earlier studies by the authors (Ognik et al., 2016; Cholewińska et al., 2018; Ognik et al., 2019; Ognik et al., 2020; Cholewińska et al., 2023; Majewski et al., 2023; Juśkiewicz et al., 2024; Marzec et al., 2024; Marzec et al., 2025), and their physicochemical properties were previously characterised in detail by Cholewińska et al. (2018). $CuCO_3$, used as a control dietary source of Cu, was obtained from Merck KGaA (Darmstadt, Germany). A control dietary fibre source was used in the form of α-cellulose (Sigma, Poznań, Poland). The experimental dietary fibre compounds used in the experiment were: pectin (PectinE 440(I), Brouwland, Beverlo, Belgium), inulin (Frutafit Tex, Sensus, Netherlands), and psyllium (Psyllim husk powder, NaturaleBio, Rome, Italy).

Experimental protocol

All animal care and experimental procedures complied with Polish legislation concerning animal experimentation and ethical standards, as well as with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The study protocol was approved by the Local Ethics Committee for Animal Experiments in Olsztyn (Approval No. 19/2021; Olsztyn, Poland).

The animals were obtained from the laboratory rat breeding facility (breeder register No. 051) at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences (IARFR PAS) in Olsztyn, Poland. Healthy 9-week-old outbred male Wistar rats (Cmdb:Wi CMDB) were fed for 6 weeks a standard semi-purified rat diet containing two levels of CuNPs (6.5 and 13 mg/kg diet, respectively) in combination with different types of dietary fibre. All diets were prepared in the laboratory using high-quality components, including casein as the main protein source, rapeseed oil as the fat source, and maize starch as the main energy source (Table 1). The control dietary fibre, α-cellulose, was added at 8% of the diet, while the experimental fibres, inulin (prebiotic), psyllium (bulking), and pectin (viscous), were added at 6% of the diet in place of cellulose. Depending on the dietary treatment, the corresponding dietary fibres were added as dry powdered preparations directly to the diet formulations.

Table 1. The composition of experimental diets administered to rats for 6 weeks (diet composition: % (g/100 g))

Indices	C	CH	CN	CNH	PN	PNH	JN	JNH	SN	SNH
Casein[1]	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Cellulose[2]	8.0	8.0	8.0	8.0	2.0	2.0	2.0	2.0	2.0	2.0
Pectin					6	6				
Inulin							6	6		
Psyllium									6	6
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Rapeseed oil	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Cholesterol	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin mix[3]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix[4]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Maize starch[5]	64.0	64.0	64.0	64.0	64.0	64.0	64.0	64.0	64.0	64.0
Calculation: Cu from, mg/kg										
$CuCO_3$	6.5	13	0	0	0	0	0	0	0	0
CuNPs	0	0	6.5	13	6.5	13	6.5	13	6.5	13

[1] casein preparation: %: crude protein 89.7, crude fat 0.3, ash 2.0, water 8.0; [2] α-cellulose (SIGMA, Poznan, Poland), the main source of dietary fibre; [3] AIN-93G-VM (Reeves, 1997), g/kg mix: nicotinic acid 3.0, Ca pantothenate 1.6, pyridoxine-HCl 0.7, thiamine-HCl 0,.6 riboflavin 0.6, folic acid 0.2, biotin 0.02, vitamin B_{12} (cyanocobalamin, 0.1% in mannitol), 15.0 vitamin E (all-rac-α-tocopheryl acetate, 500 IU/g), vitamin A 0.8 (all-trans-retinyl palmitate, 500000 IU/g), vitamin D_3 0.25 (cholecalciferol, 400000 IU/g), vitamin K_1 0.075 (phylloquinone), powdered sucrose 974.655; [4] in the experimental treatments with CuNPs in the MX $CuCO_3$ was not included. For the safety of the technician preparing the experimental diets, the CuNPs preparation was added as an emulsion with dietary rapeseed oil. This procedure has been successfully used in our previous experiments; [5] maize starch preparation: %: crude protein 0.6, crude fat 0.9, ash 0.2, total dietary fibre 0, water 8.8; groups C and CH – fed a control diet with tested and enhanced Cu content in the mineral mixture (6.5 and 13 mg/kg from $CuCO_3$, respectively) with 8% of cellulose as dietary fibre source; groups CN and CNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 8% of cellulose dietary fibre source; groups PN and PNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of pectin dietary fibre source; groups JN and JNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of inulin dietary fibre source; groups SN and SNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of psyllium dietary fibre source

The control diet contained a mineral mixture providing Cu at either the basal dose (6.5 mg/kg diet) or the twofold higher dose (13 mg/kg diet), with $CuCO_3$ as the Cu source. Experimental diets containing the conventional Cu form $CuCO_3$ were prepared by thoroughly mixing appropriately weighed amounts of the salt with the mineral premix, which was subsequently incorporated into the basal diet. For experimental diets containing CuNPs (6.5 and 13 mg/kg diet), direct addition to the mineral premix was not feasible due to the fine particulate nature of the material. Instead, after weighing, the nanoparticles were dispersed in an appropriate amount of canola oil, a component of the basal diet, to prevent loss of this micronutrient. The resulting suspension was then added to the basal diets previously mixed with the mineral premix containing the remaining essential trace elements. All components were subsequently thoroughly mixed to ensure uniform nanoparticle distribution throughout the entire batch. Directly before feeding, each diet was remixed to maintain homogeneity. The average dietary intake in the experimental groups during the experimental period ranged from 17.9 to 19.1 g per animal per day. Detailed intake data have been reported previously (Cholewińska et al., 2023). The experimental design comprised 10 groups, 10 animals each.

Sample collection and analyses

Before the end of the experiment, the rats were fasted for 8 h with free access to water. Next, they were anaesthetised by intraperitoneal injection of ketamine (K) and xylazine (X) in 0.9% NaCl (100 and 10 mg/kg body weight, respectively), in accordance with guidelines for anaesthesia and euthanasia of laboratory rodents. Following laparotomy, blood samples were collected from the caudal vena cava into heparinised tubes and EDTA-coated tubes. The animals were subsequently euthanised by cervical dislocation following Annex IV to Directive 2010/63/EU (n = 10 per group). Blood plasma was obtained by allowing whole blood collected into heparinised tubes to clot, followed by low-speed centrifugation (350 *g*, 10 min, 4 °C). Plasma samples were stored at −80 °C until analysis.

In whole blood, the following haematological parameters were determined using an ABACUS Jr VET Analyzer (DIATRON MI PLC, Budapest, Hungary): total white blood cell (WBC) count,

lymphocyte (LYM) count and percentage, medium-sized cell (MID) count and percentage, neutrophils (NEU) count and percentage, red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet (PLT) count, platelet percentage (PCT), mean platelet volume (MPV), and platelet distribution width (PDWc). In plasma, the concentrations of selected immune parameters were measured: immunoglobulins A, G, and M (IgA, IgG, and IgM), interleukin-6 (IL-6), interleukin-2 (IL-2), tumour necrosis factor a (TNF-α), and C-reactive protein (CRP). These measurements were performed using commercial enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource Inc., San Diego, CA, USA), strictly following the manufacturer's instructions without modifications. Absorbance was read at 450 nm using a SunriseTM ELISA reader (Tecan Group Ltd., Männedorf, Switzerland). Plasma albumin (ALB) concentration was determined using an automatic biochemical analyser (Plasma Diagnostic Instruments Horiba, Kyoto, Japan).

Statistical analysis

Data were analysed using STATISTICA, version 12.0 (StatSoft Corp., Krakow, Poland) to determine whether variables differed between treatment groups. A two-way ANOVA was applied to assess the effects of the main factors, i.e., CuNPs inclusion level (L, 6.5 mg/kg and H, 13 mg/kg) and dietary fibre type (cellulose, pectin, inulin, and psyllium), as well as their interactions. When ANOVA indicated significant treatment effects, group means were compared using Duncan's multiple-range test. In addition, each experimental group receiving the lower level of CuNPs (L) was compared with the corresponding control group (C; diet containing 6.5 mg/kg Cu from $CuCO_3$ with cellulose as the main fibre) using a t-test. Experimental groups receiving the higher CuNPs dose (H) were similarly compared with the corresponding control group (CH; diet containing 13 mg/kg Cu from $CuCO_3$ with cellulose) using a t-test. Data were checked for normality prior to analysis, and differences were considered significant at $P \leq 0.05$.

Results

Comparison of CN, PN, JN, SN vs. C group

The experimental groups receiving the lower CuNPs dose (6.5 mg/kg) were compared with the corresponding C group – 6.5 mg/kg Cu from $CuCO_3$ and 8% cellulose as the dietary fibre source. The CN group was fed a diet supplemented with 6.5 mg/kg CuNPs and 8% cellulose as the dietary fibre source. In the PN group, the diet contained 6.5 mg/kg CuNPs, with 2% cellulose and 6% pectin as the fibre source. The JN group received 6.5 mg/kg CuNPs with 2% cellulose and 6% inulin, while the SN group received 6.5 mg/kg CuNPs with 2% cellulose and 6% psyllium.

Significant differences between groups were assessed using one-way ANOVA ($P = 0.05$). Rats in the SN group showed higher WBC and lymphocyte counts in the blood (Table 2). Compared to the C group, rats in the JN and SN groups had lower plasma concentrations of IgM, IL-6, and TNF-α. On the other hand, IgA levels were reduced in the CN, JN, and SN groups relative to the control. IL-2 concentrations were elevated in the CN and PN groups compared with the C group. CRP levels were increased in the CN group and decreased in the PN group relative to the control (Table 4).

Comparison of CNH, PNH, JNH, SNH vs. CH group

The experimental groups receiving the higher CuNPs dose (13.0 mg/kg) were compared with the corresponding CH control group, fed a diet containing 13.0 mg/kg Cu from $CuCO_3$ and 8% cellulose as the dietary fibre source. The CNH group was fed a diet supplemented with 13.0 mg/kg CuNPs and 8% cellulose. In the PNH group, the diet contained 13.0 mg/kg CuNPs with 2% cellulose and 6% pectin. The JNH group received 13.0 mg/kg CuNPs with 2% cellulose and 6% inulin, while the SNH group was administered 13.0 mg/kg CuNPs with 2% cellulose and 6% psyllium.

Compared to the CH group, rats in the SNH group showed a decrease in mean corpuscular haemoglobin (MCH) and an increase in red cell distribution width (RDWc) and mean platelet volume (MPV) (Table 3). Plasma levels of IgA, IgM, IL-2, and TNF-α were lower in the PNH, JNH, and SNH groups compared to the control CH group. IgG concentrations were reduced in the PNH group, while CRP levels were decreased in the CNH group relative to CH group.

Two-way ANOVA

Two-way analysis of variance showed that, compared to cellulose (standard fibre), supplementation with pectin (P) or inulin (J) reduced WBC and LYM counts in rat blood ($P = 0.015$ and $P = 0.011$, respectively; Table 2). No significant effects

Table 2. White blood cell parameters in the blood of rats fed experimental diets (n = 10 per group)*

Indices	WBC, 10^3/µl	LYM, 10^3/µl	MID, 10^3/µl	NEU, 10^3/µl	LYM, %	MID, %	NEU, %
Control C	5.73	4.61	0.310	0.811	80.8	5.32	14.2
Control CH	5.75	4.51	0.366	0.872	78.3	6.27	15.4
2-way ANOVA:							
CN	6.86	5.50	0.393	0.970	80.5	5.54	14.0
CNH	6.45	5.27	0.272	0.908	81.3	4.37	14.2
PN	5.65	4.55	0.294	0.808	80.3	5.34	14.4
PNH	5.96	4.71	0.397	0.849	79.0	6.65	14.3
JN	5.60	4.48	0.273	0.845	79.4	5.32	15.0
JNH	5.91	4.66	0.410	0.843	78.4	7.22	14.4
SN	6.69#	5.56#	0.318	0.815	83.1	4.73	12.2
SNH	6.64	5.35	0.287	1.00	80.4	4.17	15.5
SEM	0.115	0.103	0.019	0.027	0.599	0.310	0.386
CuNPs dose (D)							
L (6.5 mg/kg)	6.20	5.02	0.320	0.860	80.8	5.23	13.9
H (13 mg/kg)	6.26	5.00	0.344	0.903	79.7	5.64	14.6
P-value	0.811	0.979	0.574	0.461	0.432	0.567	0.406
Fibre type (F)							
C (cellulose)	6.65[a]	5.39[a]	0.333	0.939	80.9	4.96	14.1
P (pectin)	5.80[b]	4.63[b]	0.346	0.829	79.6	6.00	14.4
J (inulin)	5.80[b]	4.60[b]	0.347	0.849	78.8	6.34	14.7
S (psyllium)	6.67[a]	5.46[a]	0.303	0.909	81.7	4.45	13.8
P-value	0.015	0.011	0.881	0.520	0.451	0.205	0.906
Interaction D×F							
P-value	0.675	0.842	0.127	0.497	0.846	0.332	0.374

* dietary treatments used in the experimental feeding period: groups C and CH – fed a control diet with tested and enhanced Cu content in the mineral mixture (6.5 and 13 mg/kg from $CuCO_3$, respectively) with 8% of cellulose as dietary fibre source; groups CN and CNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 8% of cellulose dietary fibre source; groups PN and PNH, fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of pectin dietary fibre source; groups JN and JNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of inulin dietary fibre source; groups SN and SNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of psyllium dietary fibre source; L – treatment (n = 40) with dietary CuNPs 6.5 mg/kg dose; H – treatment (n = 40) with dietary CuNPs 13 mg/kg dose; C – treatment (n = 20) with cellulose as dietary fibre; P – treatment (n = 20) with pectin as dietary fibre; J – treatment (n = 20) with inulin as dietary fibre; S – treatment (n = 20) with psyllium as dietary fibre; WBC – total white blood cells, LYM – lymphocytes, MID – mid-sized cells, NEU – neutrophils, SEM – pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n = 100); [ab] – mean values within a column with unlike superscript letters are shown to be significantly different ($P < 0.05$); differences among the groups (CN, CNH, PN, PNH, JN, JNH, SN, SNH) are indicated with superscripts only in the case of a statistically significant interaction D×F ($P < 0.05$). Additionally, each experimental group fed CuNPs at 6.5 mg/kg (CN, PN, JN, SN) and at 13 mg/kg (CNH, PNH, JNH, SNH) was compared with the respective control groups (C and CH) using a t-test; # indicates a significant difference between CN, PN, JN, SN versus the C group, whereas no significant differences were observed for the 13 mg/kg groups (CNH, PNH, JNH, SNH) versus the CH group

of the main factors, i.e., dietary fibre type or CuNPs dose, were found on red blood cell and platelet indices (Table 3). Inclusion of inulin (J) or psyllium (S) as the dietary fibre source in rats led to a decrease in plasma IL-6 levels ($P = 0.001$) relative to cellulose, while no such effect was observed with pectin supplementation (Table 4).

Two-way ANOVA revealed significant dose × fibre (D×F) interactions for IgM ($P = 0.008$), IgG ($P = 0.032$), IgA ($P < 0.001$), IL-2 ($P < 0.001$), TNF-α ($P = 0.004$), and CRP ($P < 0.001$), indicating that the main factors: dietary fibre type (F) and CuNPs dose (D), did not have a uniform effect on these parameters. For IgM, the D×F interaction resulted from the fact that an increased CuNPs dose, combined with cellulose or pectin supplementation, decreased IgM levels, whereas no change was observed with inulin or psyllium.

Table 3. Red blood cell and platelet parameters in the blood of rats fed experimental diets (n = 10 per group)*

Indices	RBC, 10^6/µl	HGB, g/dl	HCT, %	MCV, fL	MCH, pg	MCHC, g/dl	RDWc, %	PLT, 10^3/µl	PCT, %	MPV, fL	PDWc, %
Control C	9.06	14.6	42.6	47.0	16.1	34.2	18.9	592	0.478	8.08	35.6
Control CH	9.25	14.9	40.0	46.4	16.2	34.7	18.9	598	0.479	8.04	35.8
2-way ANOVA											
CN	9.14	14.8	42.6	46.7	16.2	34.8	19.0	596	0.442	8.23	36.3
CNH	9.25	14.6	42.8	46.7	15.9	34.1	19.0	591	0.484	8.21	36.4
PN	9.23	14.7	43.0	46.7	15.9	34.2	19.0	620	0.507	8.16	36.1
PNH	9.36	14.8	42.7	46.2	16.0	34.5	19.2	642	0.531	8.31	35.7
JN	9.23	14.7	43.5	47.1	16.0	33.9	19.3	631	0.500	8.33	36.1
JNH	9.30	14.9	43.8	46.9	16.0	34.0	18.9	641	0.449	7.95	35.7
SN	8.92	14.5	42.1	47.2	16.3	34.2	18.9	601	0.494	8.23	35.6
SNH	9.31	14.6	42.6	45.6	15.7[&]	34.2	19.3[&]	625	0.528	8.44[&]	36.5
SEM	0.043	0.062	0.328	0.132	0.055	0.101	0.056	7.096	0.008	0.039	0.109
CuNPs dose (D)											
L (6.5 mg/kg)	9.13	14.7	42.8	46.9	16.1	34.3	19.0	612	0.486	8.24	36.0
H (13 mg/kg)	9.32	14.8	43.0	46.3	15.9	34.3	19.1	626	0.497	8.22	36.1
P-value	0.055	0.490	0.590	0.066	0.094	0.997	0.530	0.417	0.571	0.822	0.815
Fibre type (F)											
C (cellulose)	9.19	14.7	42.7	46.7	16.1	34.4	19.0	593	0.463	8.22	36.3
P (pectin)	9.29	14.7	42.9	46.5	15.9	34.3	19.1	631	0.519	8.23	35.9
J (inulin)	9.30	14.9	43.8	47.0	16.0	34.0	19.1	638	0.472	8.12	35.9
S (psyllium)	9.12	14.5	42.3	46.4	16.0	34.2	19.1	613	0.511	8.34	36.1
P-value	0.500	0.344	0.104	0.518	0.863	0.564	0.941	0.227	0.101	0.401	0.586
Interaction D×F											
P-value	0.679	0.573	0.882	0.269	0.114	0.290	0.200	0.916	0.252	0.061	0.222

dietary treatments used in the experimental feeding period: groups C and CH – fed a control diet with tested and enhanced Cu content in the mineral mixture (6.5 and 13 mg/kg from $CuCO_3$, respectively) with 8% of cellulose as dietary fibre source; groups CN and CNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 8% of cellulose dietary fibre source; groups PN and PNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of pectin dietary fibre source; groups JN and JNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of inulin dietary fibre source; groups SN and SNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of psyllium dietary fibre source; L – treatment (n = 40) with dietary CuNPs 6.5 mg/kg dose; H – treatment (n = 40) with dietary CuNPs 13 mg/kg dose; C – treatment (n = 20) with cellulose as dietary fibre; P – treatment (n = 20) with pectin as dietary fibre; J – treatment (n = 20) with inulin as dietary fibre; S – treatment (n = 20) with psyllium as dietary fibre; RBC – red blood cells, HGB – haemoglobin, HCT – haematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular hemoglobin concentration, RDWc – red cell distribution width, PLT – platelet count, PCT – platelet percentage, MPV – mean platelet volume, PDWc – platelet distribution width, SEM – pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n = 100); differences among the groups (CN, CNH, PN, PNH, JN, JNH, SN, SNH) are indicated with superscripts only in the case of a statistically significant interaction D×F ($P < 0.05$). Additionally, each experimental group fed CuNPs at 6.5 mg/kg (CN, PN, JN, SN) and at 13 mg/kg (CNH, PNH, JNH, SNH) was compared with the respective control groups (C and CH) using a t-test; [&] indicates a significant difference between CNH, PNH, JNH, SNH versus the CH group, whereas no significant differences were observed for the 6.5 mg/kg groups (CN, PN, JN, SN) versus the C group

For IgG and TNF-α, the interaction results showed that only the combination of a higher CuNPs dose with pectin reduced these parameters, while other fibre types showed no effect. For IgA, the decrease occurred when the higher CuNPs dose was combined with pectin or inulin, but not with cellulose or psyllium. Regarding IL-2, the D×F interaction was due to an increase in IL-2 levels when higher CuNPs dose was combined with cellulose or psyllium, a decrease with pectin, and no effect with inulin. For CRP, the higher CuNPs dose increased CRP levels when combined with pectin, decreased it with cellulose, and had no effect with inulin or psyllium.

Table 4. Immune parameters in the blood plasma of rats fed experimental diets (n = 10 per group)

Indices	IgM, µg/ml	IgG, µg/ml	IgA, µg/ml	IL-2, ng/l	IL-6, ng/ml	TNFα, pg/ml	CRP, ng/ml	ALB, µmol/l
Control C	936	4085	6190	253	62.7	733	18.3	459
Control CH	875	4208	6277	435	59.6	696	17.1	460
2-way ANOVA:								
CN	860^{a}	4239^{a}	$5516^{a\#}$	$334^{bc\#}$	59.1	663^{a}	$22.7^{a\#}$	466
CNH	734^{b}	4379^{a}	5806^{a}	431^{a}	59.3	650^{ab}	$14.3^{cd\&}$	464
PN	888^{a}	4329^{a}	5715^{a}	$368^{b\#}$	60.7	656^{a}	$10.4^{d\#}$	459
PNH	$665^{b\&}$	$3709^{b\&}$	$3764^{bc\&}$	$312^{c\&}$	58.6	$517^{c\&}$	18.5^{bc}	451
JN	$687^{b\#}$	4090^{ab}	$4240^{b\#}$	264^{d}	$52.2^{\#}$	$548^{c\#}$	18.9^{ab}	455
JNH	$707^{b\&}$	4077^{ab}	$3380^{c\&}$	$241^{d\&}$	54.8	$587^{abc\&}$	17.3^{bc}	471
SN	$624^{b\#}$	3971^{ab}	$3766^{bc\#}$	262^{d}	$51.9^{\#}$	$522^{c\#}$	17.1^{bc}	459
SNH	$675^{b\&}$	3963^{ab}	$4237^{b\&}$	$328^{bc\&}$	52.8	$572^{bc\&}$	18.3^{bc}	445
SEM	16.190	42.448	126.20	8.568	0.753	10.812	0.509	2.243
Cu-NP dose (D)								
L (6.5 mg/kg)	765	4157	4809	307	56.0	597	17.3	460
H (13 mg/kg)	695	4032	4297	328	56.4	581	17.1	458
P-value	0.028	0.196	0.003	0.037	0.815	0.417	0.823	0.754
fibre type (F)								
C (cellulose)	797	4309	5661	382	59.2^{a}	656	18.5	465
P (pectin)	777	4019	4740	340	59.7^{a}	587	14.4	455
J (inulin)	697	4083	3810	252	53.5^{b}	568	18.1	463
S (psyllium)	650	3967	4002	295	52.4^{b}	547	17.7	452
P-value	0.003	0.068	<0.001	<0.001	0.001	0.001	0.017	0.227
Interaction D×F								
P-value	0.008	0.032	<0.001	<0.001	0.757	0.004	<0.001	0.178

dietary treatments used in the experimental feeding period: groups C and CH – fed a control diet with tested and enhanced Cu content in the mineral mixture (6.5 and 13 mg/kg from $CuCO_3$, respectively) with 8% of cellulose as dietary fibre source; groups CN and CNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 8% of cellulose dietary fibre source; groups PN and PNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of pectin dietary fibre source; groups JN and JNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of inulin dietary fibre source; groups SN and SNH – fed diets with supplementation of CuNPs (6.5 and 13 mg/kg from Cu-nanoparticles, respectively) with 2% of cellulose and 6% of psyllium dietary fibre source; L – treatment (n = 40) with dietary CuNPs 6.5 mg/kg dose; H – treatment (n = 40) with dietary CuNPs 13 mg/kg dose; Ct – reatment (n = 20) with cellulose as dietary fibre; P – treatment (n = 20) with pectin as dietary fibre; J – treatment (n = 20) with inulin as dietary fibre; S – treatment (n = 20) with psyllium as dietary IgM – immunoglobulin M, IgG – immunoglobulin G, IgA – immunoglobulin A, IL-2 – interleukin 2, IL-6 – interleukin 6, TNFα – tumor necrosis factor alpha, CRP – C-reactive protein, ALB – albumin, SEM – pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n = 100); $^{a–d}$ – mean values within a column with unlike superscript letters are shown to be significantly different ($P < 0.05$); differences among the groups (CN, CNH, PN, PNH, JN, JNH, SN, SNH) are indicated with superscripts only in the case of a statistically significant interaction D×F ($P < 0.05$). Additionally, each experimental group fed CuNPs 6.5 mg/kg (CN, PN, JN, SN) was compared with the control C group with the aid of a t-test ($^{\#}$ indicates a significant difference versus the C group); similarly, each experimental group fed CuNPs 13 mg/kg (CNH, PNH, JNH, SNH) was compared with the control CH group with the aid of a t-test ($^{\&}$ indicates a significant difference versus the CH group)

Discussion

Replacing cellulose with fermentable fibres, such as inulin or pectin, attenuated inflammation, as reflected by lower white blood cell (WBC) and lymphocyte (LYM) counts, and reduced serum interleukin-6 (IL-6) concentrations. This effect is likely associated with microbial fermentation of these fibres to short-chain fatty acids (SCFAs), mainly butyrate, acetate, and propionate. This finding is consistent with our previous studies showing that inulin effectively stimulates butyrate and propionate production, whereas pectin enhances colonic bacterial enzyme activity and maintains beneficial microbial metabolic functions (Juśkiewicz et al., 2024). Cu homeostasis is regulated by specific cellular transporters, including divalent metal transporter 1 (DMT1) and Cu transporter 1 (CTR1), which mediate Cu absorption primarily in the duodenum (Turnlund, 1998; Harris, 2001). The efficiency of this process depends on the chemical form and speciation of dietary Cu (Bost et al., 2016).

Importantly, the bioavailability and absorption of Cu in the gastrointestinal tract are determined by pH-controlled speciation and solubility. While Cu carbonate ($CuCO_3$) has low solubility at neutral pH, it undergoes rapid dissolution in acidic gastric conditions (pH 1.5–2.0), forming bioavailable Cu^{2+} ions. In the proximal small intestine, these ions precipitate as poorly soluble $Cu(OH)_2$, which results in limited and physiologically regulated absorption (Wu et al., 2021). CuNPs display significantly higher systemic bioavailability compared to $CuCO_3$. Under identical experimental conditions, Cu utilisation (retention) reached 24.6% for CuNPs versus 8.88% for $CuCO_3$ ($P < 0.001$) (Cholewińska et al., 2018). This difference arises from rapid CuNPs dissolution in acidic gastric juice, with more than 84% Cu release at pH 1.5 within 24 h (Lee et al., 2016), and from dynamic *in vivo* oxidation and transformation of metallic Cu^0 to Cu_2O and CuO, accompanied by the release of bioavailable Cu^{2+} and Cu^+ ions (Karlsson et al., 2008). Dietary amino acids further increase CuNPs dissolution and bioaccumulation, even under neutral intestinal pH conditions (Boyle et al., 2020), which indicates that Cu nanoparticles remain chemically reactive in relevant physiological environments. Consequently, the effective bioavailable dose of CuNPs is approximately twofold higher than the nominal dietary dose relative to $CuCO_3$ (Cholewińska et al., 2018). This difference should be considered when interpreting dose-response relationships and the immunological effects observed in the present study. SCFAs can bind to receptors on immune cell surfaces, inhibiting histone deacetylase activity and NF-κB signalling, thereby downregulating transcription of TNF-α, IL-6, and CRP genes. They also affect the maturation and function of antigen-presenting cells and macrophages, inducing changes in their phenotype towards an anti-inflammatory profile and supporting the development and activation of regulatory T cells (Tregs), which suppress excessive immune responses (Foey, 2011; Kim et al., 2013; Beukema et al., 2020; Kim, 2023; Sheng et al., 2023). Pectin may exert additional immunomodulatory effects through interaction with pattern recognition receptors, which inhibits LPS-induced IL-6 release from macrophages. Inulin, in turn, suppresses NF-κB pathway activity in epithelial cells and macrophages, directly reducing the production of IL-6 and other pro-inflammatory cytokines. Moreover, both fibres strengthen intestinal barrier function, as demonstrated in our earlier work (Cholewińska et al., 2023). The literature indicates that this effect occurs mainly through SCFA-mediated stimulation of epithelial cell proliferation and increased expression of tight junction proteins, which limits endotoxin translocation and secondary leukocyte activation (Blanco-Pérez et al., 2021; Li et al., 2023; Sheng et al., 2023). Our previous studies also showed that inulin supports DNA repair mechanisms in small-intestinal epithelial cells, while pectin inhibits inflammatory processes that induce apoptosis in these cells (Cholewińska et al., 2023). Additionally, pectin, through its galacturonic acid residues, can chelate metal ions, and thus reduce Cu-induced ROS formation (Lara-Espinoza et al., 2018). These complex molecular mechanisms may explain the haematological and biochemical changes occurring independently of Cu dose and form, showing the universal anti-inflammatory properties of fermentable fibres.

Partial replacement of cellulose with psyllium husk, in combination with CuNPs at the tested Cu exposure dose, increased white blood cell and lymphocyte counts, in contrast to the anti-inflammatory effects observed for inulin and pectin. This difference likely reflects the distinct physicochemical and biological properties of psyllium. Unlike highly fermentable fibres, psyllium is only partially fermented and acts mainly through gel formation and bile acid (BA) sequestration, which increases circulating BA levels and activates the farnesoid X receptor (FXR). Activation of the latter receptor may exert immunostimulatory effects in certain conditions by promoting leukopoiesis and lymphocyte proliferation. In addition, limited fermentation of psyllium results in lower SCFA production compared to inulin or pectin, which reduces SCFA-mediated anti-inflammatory signalling through inhibition of the NF-κB pathway. Psyllium may also alter the gut microbiota composition in a manner that transiently increases immune cell production. Elevated BA flow can induce stress responses that mobilise WBC (Bretin et al., 2023). Moreover, although the gelling viscosity of psyllium may slow intestinal passage, it does not chelate Cu ions as effectively as the charged groups present in pectin. Prolonged transit may therefore extend mucosal exposure to CuNPs and lead to mild immune activation. In contrast, Bretin et al. (2023) reported that psyllium-enriched diets protected against colonic inflammation through mechanisms independent of SCFA or IL-22 signalling and requiring only limited microbiota involvement. In human studies, psyllium has shown inconsistent effects on inflammatory markers, with some trials reporting no changes in CRP or IL-6 concentrations. These findings indicate that

its impact on immune parameters may vary with Cu dose or form (King et al., 2008).

The results of the present study demonstrated that partial replacement of cellulose with inulin or psyllium husk in a diet containing CuNPs at the experimental Cu exposure dose (6.5 mg Cu/kg) reduced plasma levels of IgM, IL-6, and TNF-α, which indicates attenuation of both humoral and innate inflammatory responses. Moreover, supplementation with pectin, inulin, or psyllium husk, combined with a two fold higher CuNPs dose (13 mg Cu/kg), intensified this effect and additionally reduced IgA and IL-2 levels. It can be hypothesised that the increased production of butyrate and propionate during fermentation of inulin and pectin inhibited histone deacetylases, thereby limiting immunoglobulin class-switch recombination and consequently decreasing IgA and/or IgM secretion (Foey, 2011). This mechanism of restricted class switching may also account for the reduced plasma IgG levels observed in rats fed pectin together with the high CuNPs dose. Concurrently, SCFA signalling may inhibit NFκB and AP-1 activity in T lymphocytes, downregulating IL-2 transcription and helper T cell cytokine production (Foey, 2011). Psyllium may further contribute to this effect by forming viscous gels that delay antigen absorption and reduce interactions between pathogen-associated molecular patterns (PAMPs) and Toll-like receptors (TLRs) on immune cells, further reducing TNF-α release (Bretin et al., 2023). It is also plausible that increased uptake of CuNPs at the higher dietary dose directly affects dendritic cell maturation and cytokine profiles, promoting regulatory immune phenotypes and reinforcing anti-inflammatory signals derived from dietary fibres (Dürholz et al., 2020).

Replacing inorganic Cu with CuNPs at the experimental dose in rats fed a cellulose-only fibre diet decreased plasma IgA concentrations and was accompanied by elevated C-reactive protein (CRP) levels.

It should be noted that metallic CuNPs administered in the diet may undergo partial oxidation and dissolution under physiological gastrointestinal conditions, resulting in the release of Cu^{2+} ions, as well as the formation of secondary agglomerates. Numerous studies have demonstrated that CuNPs are not chemically inert *in vivo* and may undergo dynamic transformations depending on pH, redox potential, and the presence of biological ligands, such as bile salts, digestive enzymes, and microbial metabolites. Consequently, the observed immunological and inflammatory effects likely reflect the combined action of nanoparticulate Cu and released ionic Cu rather than a response to intact metallic nanoparticles alone. This dual exposure may potentiate oxidative stress and inflammatory signalling, particularly in the absence of fermentable fibre-derived SCFAs that can counteract Cu-induced ROS generation (Karlsson et al., 2008; Studer at al., 2010). The decrease in plasma IgA concentrations suggests that the increased bioavailability of CuNPs may directly impair mucosal B-cell function, possibly through oxidative stress and apoptosis of lamina propria plasma cells. This interpretation is consistent with murine inhalation models, in which CuO nanoparticles activated immune cells while reducing the viability of antibody-secreting cells (Tulinska et al., 2022). The concomitant rise in CRP level reflects an acute-phase response resulting from hepatic synthesis in reaction to nanoparticle-induced systemic inflammation and ROS generation. Similar effects were reported in spontaneously hypertensive rats exposed to CuO nanoparticles, which showed increased serum CRP levels (Wang et al., 2022). These divergent responses, i.e., IgA suppression together with CRP elevation, indicate dual effects of CuNPs. Dietary CuNPs may increase ROS formation, which activates hepatic NF-κB and stimulates CRP synthesis, while simultaneously inducing endoplasmic reticulum stress in B cells and restricting immunoglobulin class switching to IgA. This mechanism links a systemic acute-phase response with local immunosuppression. Interestingly, rats receiving cellulose with the high CuNPs dose displayed reduced plasma CRP levels, which may be explained by oxidative hormesis and changes in hepatic redox signalling pathways. In the absence of fermentable SCFA precursors, elevated nanoparticle concentrations may induce moderate oxidative stress that activates the Nrf2 pathway in hepatocytes. This activation stimulates the expression of antioxidant enzyme genes (e.g., HO-1, NQO1) and concurrently suppresses NF-κB-dependent acute-phase protein synthesis, including CRP, as an adaptive anti-inflammatory response (Al-Ruwad et al., 2024). Moreover, under low intestinal fermentation conditions, reduced proinflammatory cytokine signalling, especially IL-6-mediated hepatocyte stimulation, may further inhibit CRP production despite increased CuNPs bioaccumulation (Sutunkova et al., 2023).

The dietary combination of psyllium husk with a high CuNPs dose also altered erythrocyte and platelet indices, indicative of mild haemolytic anaemia and reactive thrombopoiesis. Mean corpuscular haemoglobin (MCH) decreased, suggesting reduced haemoglobin concentration per

erythrocyte, likely due to impaired haemoglobin synthesis associated with micronutrient imbalance and oxidative damage (Tesser et al., 2020). At the same time, higher red cell distribution width (RDWc) reflects elevated anisocytosis caused by premature release of erythrocytes into the circulation to compensate for accelerated removal of damaged cells. Similar phenomena have been observed in rodent models exposed to CuNPs, in which oxidative membrane damage accelerated erythrocyte fragmentation (Karlsson et al., 2013). An increase in mean platelet volume (MPV) further confirms reactive thrombocytosis associated with systemic inflammatory and oxidative stimuli. Platelet progenitor cells produce larger and more reactive platelets under stress to maintain haemostasis during intravascular haemolysis. The gel-forming matrix of psyllium husk may exacerbate these effects by altering Cu absorption kinetics, causing local fluctuations in Cu availability that impair red blood cell maturation and increase CuNPs-induced ROS generation.

Although the present findings provide valuable information on the interactions between dietary fibre types and CuNPs in relation to immune function, several limitations should be noted. First, the rodent model, despite its wide use, does not fully reproduce human gastrointestinal physiology or microbiome diversity, which could potentially limit the direct extrapolation of these results to humans. Second, the CuNPs used in this study (40 nm) represent only one nanoparticle formulation. Biological effects may differ substantially depending on particle size and surface characteristics. Future research should investigate doseresponse relationships over a broader range of CuNPs concentrations and particle sizes, as well as the long-term effects of their interaction with various dietary fibres on the immunological status of experimental animals. Additionally, detailed mechanistic studies are required to elucidate the molecular pathways responsible for the modulatory properties of different fibre types in the presence of CuNPs. Such research will significantly advance our understanding of these complex interactions and facilitate the development of targeted nutritional strategies.

Conclusions

During supplementation with the elevated copper(II) carbonate ($CuCO_3$) dose, pectin exerted the most beneficial effects on immunological parameters and demonstrated stronger anti-inflammatory activity compared to inulin or psyllium husk. Replacement of the traditional copper source with nanoparticles, even at the lower experimental dose, exacerbated inflammation and impaired immune function in rats. Partial substitution of cellulose with inulin or pectin in the rats' diet mitigated the adverse effects induced by copper nanoparticles (CuNPs) regardless of dose, with inulin showing the strongest anti-inflammatory effect of the alternative dietary fibres.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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