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Haematological Evaluation of Albino Rats Fed with Pleurotus ostreatus Cultivated on Diverse Organic Substrates

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Abstract
This study evaluates the haematological impacts of Pleurotus ostreatus (oyster mushroom) cultivated on various substrates and integrated into the diets of albino rats (Rattus norvegicus). Using a controlled six-week feeding trial, sixty-three Sprague Dawley rats were grouped and administered diets incorporating P. ostreatus cultivated on compost and vermicompost substrates derived from different animal manures and lignocellulosic materials. Standard haematological indices—PCV, ESR, RBC, WBC, Hb, and differential WBC counts—were assessed post-trial to determine physiological responses. Results indicated substrate-specific variations in blood parameters. Notably, rats fed mushrooms cultivated on poultry droppings exhibited elevated RBC and Hb levels, suggesting improved erythropoiesis, while goats' dung substrates correlated with higher WBC counts, potentially reflecting immunomodulatory effects. The control group, which did not consume mushroom-infused diets, showed comparatively lower haematological values. These findings underscore the influence of cultivation substrate on the nutritional and therapeutic properties of P. ostreatus, with implications for its application in functional animal feed development. Further research is recommended to elucidate the biochemical mechanisms driving these effects.

Keywords: Albino rats, Compost substrates, Functional feed, Haematology, Pleurotus ostreatus.

Introduction

The rising global demand for functional foods and alternative protein sources has renewed scientific interest in the nutritional and therapeutic potential of edible mushrooms. Among these, *Pleurotus ostreatus*—commonly known as oyster mushroom—has emerged as a promising candidate due to its rich profile of essential amino acids, vitamins, antioxidants, and immunomodulatory compounds (Bisen et al., 2010). Beyond its culinary appeal, *P. ostreatus* has demonstrated bioactivities that support cardiovascular, hepatic, and hematopoietic health (Gunde-Cimerman, 1999; Alam et al., 2008). As the substrate on which a mushroom is cultivated can significantly influence its biochemical composition, attention has shifted toward understanding how growth media affect its nutritive and medicinal qualities. Agricultural residues, particularly lignocellulosic biomass supplemented with animal waste, offer an ecologically sound and nutritionally enriching base for mushroom cultivation (Girmay et al., 2016). However, limited studies have systematically examined the downstream physiological effects of consuming such mushrooms, especially in the context of haematological health.

Haematological parameters serve as sensitive indicators of systemic response to dietary inputs, often reflecting immunological, erythropoietic, and metabolic adjustments (Olayemi et al., 2007). Consequently, evaluating the haematological impact of *P. ostreatus* cultivated on diverse organic substrates could offer insight into its functional feed potential. This study investigates how dietary incorporation of *P. ostreatus*, grown on compost and vermicompost substrates enriched with various animal manures, affects the blood profile of albino rats. By assessing parameters such as packed cell volume (PCV), erythrocyte sedimentation rate (ESR), haemoglobin concentration, and differential white cell counts, we aim to elucidate the nutritional and therapeutic implications of substrate-specific mushroom consumption.

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Materials and Methods

i. Experimental Animals and Ethical Considerations

Sixty-three healthy albino rats (*Rattus norvegicus*, Sprague Dawley strain), four weeks old and of both sexes, were obtained from the Animal House, Department of Microbiology, Federal University of Technology, Akure, Nigeria. The animals were randomly assigned into five groups, each comprising three replicates of three rats (n=9 per group). They were housed in well-ventilated wooden cages lined with sawdust and maintained under controlled environmental conditions: temperature 25 ± 5°C, relative humidity of 60–70%, and a 12-hour light/dark cycle. The rats were allowed a one-week acclimatization period before dietary intervention began. All experimental protocols adhered to institutional and international guidelines for the ethical treatment of laboratory animals (National Research Council, 2011).

ii. Feed Formulation and Diet Preparation

The mushroom-based diets were formulated following modifications of the protein quality evaluation model proposed by del Toro et al. (2006). Three test diets were developed: one incorporating *Pleurotus ostreatus* cultivated on compost substrates, another using vermicompost-grown mushrooms, and a third employing a soya bean-based reference diet. A protein-free diet was also prepared for baseline comparison. The base components included cellulose, corn starch, sucrose, methionine, corn oil, and vitamin-mineral premix. The mushroom substrates were sourced from *P. ostreatus* cultivated on wood dust of *Pycnanthus angolensis* and *Spondias mombin*, each amended with distinct animal manures (cow, goat, poultry, and sheep dung). All ingredients were homogenized and pelletized under sterile conditions.

Table 1: Feed formulation for the evaluation of protein quality (g/1000g)

Components	Diets		
	Protein free diet(g)	Soya bean Composed diet(g)	Mushroom Composed Diet (g)
Cellulose	40.0	40.0	40.0
Sucrose	100.0	100.0	100.0
Corn oil	40.0	40.0	40.0
Min.mix/Vit mix	50.0	50.0	50.0
Methionine	4.0	4.0	4.0
Soya bean meal	-	250.0	-
Mushroom	-	-	125.0
Corn Starch	766.0	516.0	641.0
Total	1000	1000	1000

Diets were prepared according to del Toro *et al.* (2006)

iii. Animal Grouping and Feeding

Each dietary group was fed ad libitum with the assigned diet for six weeks. Table water with National Agency for Food and Drug Administration and Control (NAFDAC) registration (No. 01-7155) was provided freely. The physiological status of animals was not factored into the grouping design, allowing for a randomized distribution. Feed intake and weight gain were monitored daily and weekly, respectively, using a digital electronic balance (Gilbertini, Italy).

iv. Haematological Analysis

At the end of the feeding trial, animals were sacrificed via cervical dislocation, and whole blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes. Haematological indices were assessed at the Animal Production and Health Laboratory of the same university. The parameters measured included erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb), and WBC differential.

- ESR was determined using the Wintrobe method by measuring the fall in red cells after one hour (Dacie & Lewis, 2001).
- PCV was assessed using a micro-haematocrit centrifuge at 12,000 rpm for 5 minutes, followed by quantification with a haematocrit reader.
- RBC and WBC counts were obtained using standard hemocytometer methods after appropriate dilution (Cheesbrough, 2006).
- Haemoglobin concentration was estimated using the cyanmethemoglobin method with Drabkin's reagent, compared against a prepared standard using a spectrophotometer with a 624 nm filter.

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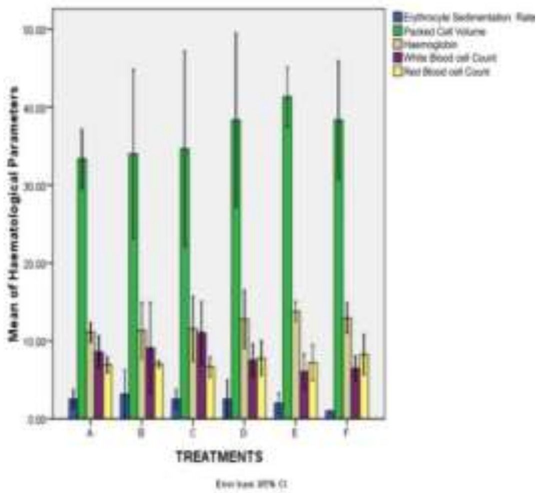
- Differential WBC counts were performed after Giemsa staining, distinguishing neutrophils, eosinophils, basophils, lymphocytes, and monocytes under oil immersion microscopy.

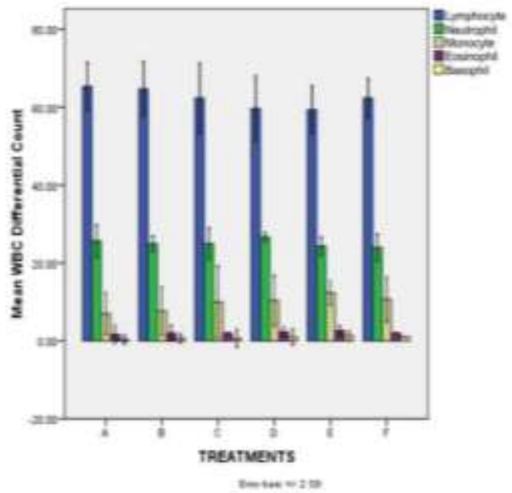
v. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA), and post-hoc comparisons were made using Duncan's Multiple Range Test. All statistical analyses were conducted using SPSS version 14 on Microsoft Windows 7. Results were expressed as mean ± standard deviation, and significance was set at $p < 0.05$.

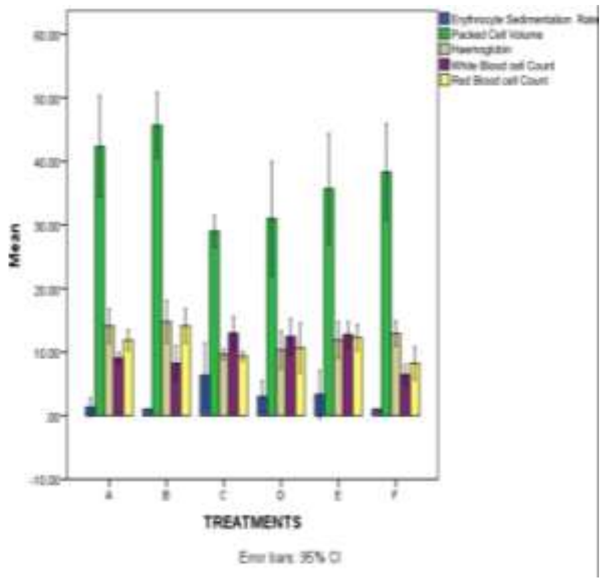
Results



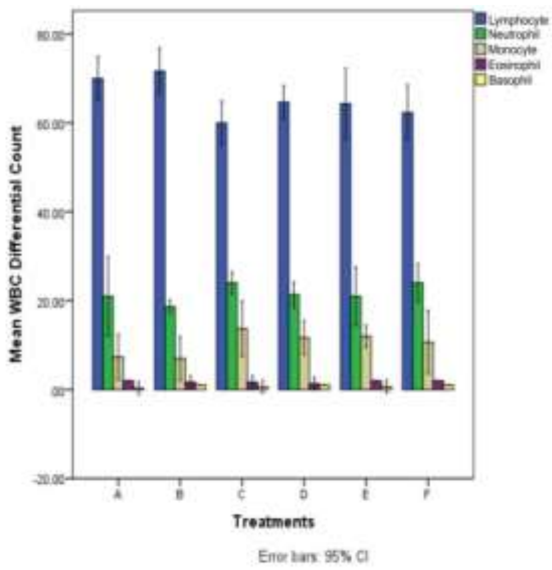
a. Mean of Haematological Parameters in Composting Substrates



b. Mean of WBC Differential Count in Composting Substrates



c. Mean of Haematological Parameters in Vermicomposting Substrates



d. Mean of WBC Differential Count in Vermicomposting Substrates

Figures 1a-d. Haematology result of rats fed with *Pleurotus ostreatus* cultivated on wood dust of *Pycnanthus angolensis* with animal wastes (A- cow dung, B- goat dung, C- poultry droppings, D- sheep dung, E- without dung F- experimental rat(control).

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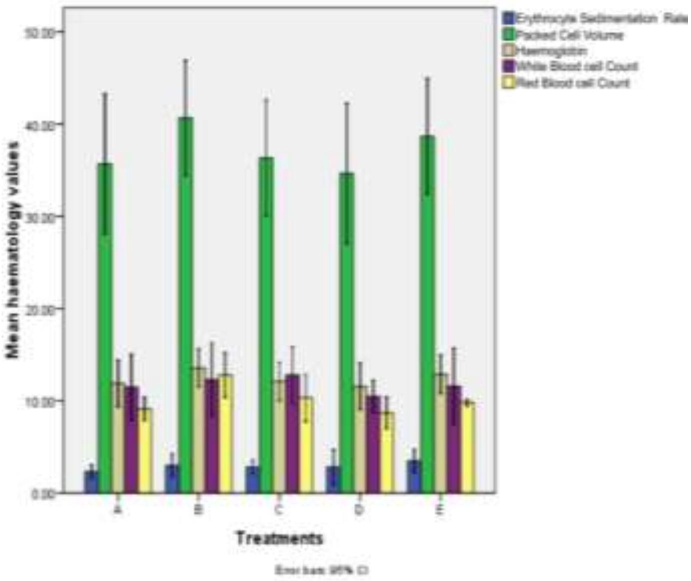

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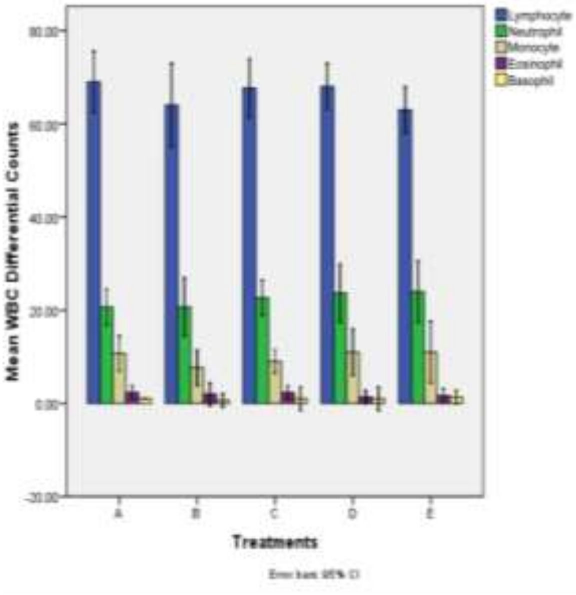
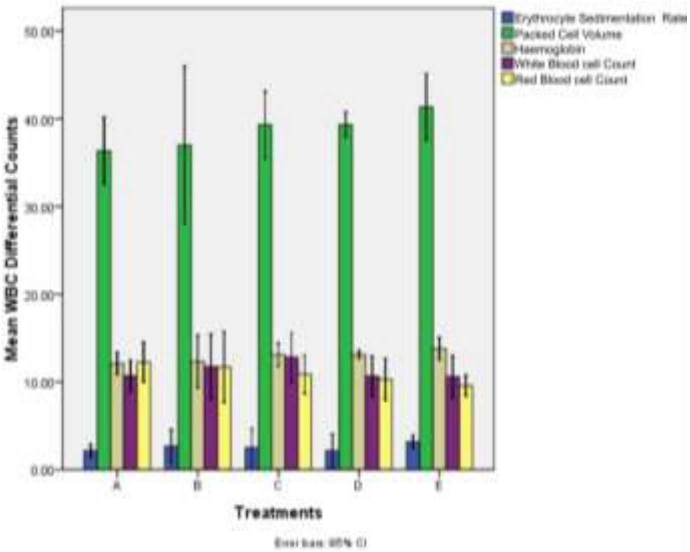
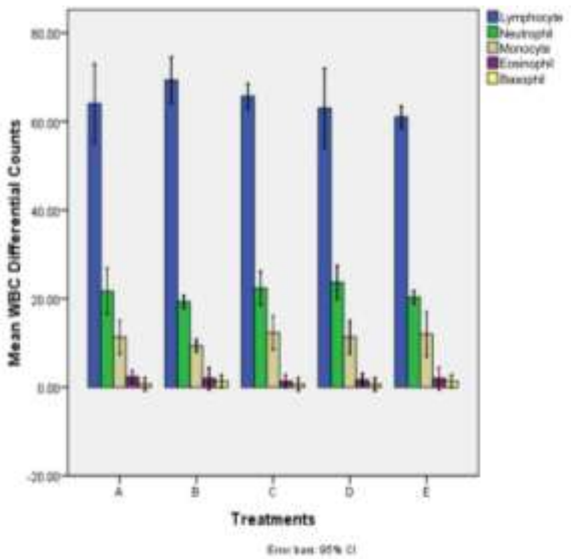


b. Mean of WBC Differential Count in Composting Substrates



c. Mean of Haematological Parameters in Vermicomposting Substrates



d. Mean of WBC Differential Count in Vermicomposting Substrates

Figure 2a-d. Haematology result of rats fed with *Pleurotus ostreatus* cultivated on wood dust of *Spondias mombin* with animal wastes. (A- cow dung, B- goat dung, C- poultry droppings, D- sheep dung, E- without dung.

i. General Health and Weight Gain

Throughout the six-week dietary intervention, all experimental groups maintained good physical condition without overt signs of morbidity. Weight monitoring revealed a gradual increase in body mass across all groups, although the extent of weight gain varied with the type of dietary supplementation. Rats fed *Pleurotus ostreatus* cultivated on poultry droppings showed the highest mean weight gain, followed by those fed mushrooms from goat and cow dung substrates. The control group, maintained on a protein-free diet, displayed minimal weight

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gain, consistent with impaired nutrient absorption, which corroborates earlier observations by Olorunfemi et al. (2012) on protein-deficient feeding regimes.

ii. Core Haematological Parameters

Significant differences ($p < 0.05$) were observed in the core haematological indices among dietary groups. Rats consuming *P. ostreatus* cultivated on poultry manure exhibited notably higher red blood cell (RBC) counts and haemoglobin (Hb) concentrations than all other groups, including the soya bean control. This enhancement in erythropoietic indices suggests a potential haematinic effect of mushrooms grown on nutrient-rich substrates, consistent with findings by Badu et al. (2011), who reported improved haematological values following mushroom-enriched diets.

Packed cell volume (PCV) levels followed a similar trend, with the highest values recorded in the poultry manure group, indicating enhanced oxygen-carrying capacity and hematocrit stability. Conversely, rats fed mushrooms cultivated without manure supplementation displayed modest haematological responses, underscoring the role of substrate composition in determining the bioactivity of *P. ostreatus*.

iii. White Blood Cell and Differential Counts

Total white blood cell (WBC) counts were significantly elevated in groups fed mushrooms grown on goat and sheep dung substrates, suggesting a possible immunostimulatory effect. This aligns with the work of Zhang et al. (2007), who noted increased leukocyte production in response to mushroom polysaccharides. Differential WBC analysis revealed substrate-specific trends: neutrophil and monocyte percentages were highest in the goat dung group, while lymphocyte counts were more elevated in rats fed with mushrooms cultivated on poultry droppings.

Interestingly, the control group showed comparatively lower WBC counts across all parameters, a pattern likely due to the absence of immune-boosting dietary components. The observed differential shifts reflect a systemic adaptation to bioactive constituents such as β-glucans and phenolics within the mushroom matrix, as influenced by substrate biochemistry (Wasser, 2011).

iv. Comparative Analysis of Compost vs. Vermicompost Substrates

Between compost and vermicompost substrates, the vermicompost variants generally yielded better haematological outcomes. Rats fed mushrooms grown on vermicomposted poultry and goat manure demonstrated superior RBC, Hb, and WBC values compared to their compost-fed counterparts. This finding suggests enhanced nutrient assimilation in vermicompost systems, possibly due to microbial conversion of organic matter into more bioavailable forms, consistent with the hypothesis proposed by Edwards et al. (2004).

Discussion

The current investigation demonstrates that dietary supplementation with *Pleurotus ostreatus*, particularly when cultivated on nutrient-enriched organic substrates, positively influences key haematological parameters in albino rats. These outcomes underscore the critical role of substrate composition in modulating the functional properties of edible mushrooms.

The observed elevation in red blood cell count, haemoglobin concentration, and packed cell volume among rats fed mushrooms grown on poultry and goat dung substrates is biologically significant. These findings suggest enhanced erythropoiesis, potentially mediated by iron, B-complex vitamins, and hematinic compounds absorbed by the mushroom from manure-rich substrates (Bano & Rajarathnam, 1988). Poultry droppings, in particular, are known to be rich in nitrogen and trace minerals, which may have facilitated the biosynthesis of erythropoietic cofactors during mushroom growth (Gbolagade et al., 2006).

This study aligns with earlier work by Badu et al. (2011), who reported that *P. ostreatus* supplementation improved erythrocyte parameters in diabetic models. The mechanism likely involves the mushroom's content of polysaccharides and antioxidants, which reduce oxidative damage to erythrocyte membranes and support marrow activity (Wasser, 2011). The immunological impact, as evidenced by elevated white blood cell counts and differential shifts—particularly increased lymphocyte and monocyte levels—suggests that mushrooms cultivated on goat and sheep dung possess immune-modulating potential. These effects may be attributed to β-glucans and other bioactive compounds inherent in mushrooms, whose synthesis may be upregulated by nutrient-rich substrates (Zhang et al., 2007). The heightened WBC values observed in the goat dung-fed group imply that certain microbial or prebiotic components in the manure may further enhance immune response via gut-immune axis activation (Cheung & Ng, 2006).

Furthermore, the superior haematological outcomes in vermicompost-fed groups compared to their compost counterparts indicate a more efficient nutrient bioconversion in vermiculture systems. Earthworm digestion

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facilitates the breakdown of organic matter into more bioavailable micronutrients, which are subsequently absorbed by the growing mushrooms (Edwards et al., 2004). The albino rats fed with *P. ostreatus* harvested from composted and vermicomposted wood dusts were as healthy as those fed with normal feed diet without mushrooms. The increase in weights of albino rats fed with *P. ostreatus* in this work further affirm the nutritional quality of this mushroom that is cultivated on the various substrates prepared. It agrees with Ekundayo *et al*. (2014), Edet *et al.* (2010) where increase in weights of albino rats fed with P. *ostreatus and P. pulmonarius* indicated growth.

The increase in (WBC) of rats fed with mushroom on composted and vermicomposted substrates of *Pycnanthus angolensis* and *Spondias numbin* can be attributed to the presence of certain amount of antinutrients in the mushroom, though the level is safe for consumption since the increase is not beyond the normal range for albino rat.(WBC 6.6-12.6 x $10^3/mm^3$)
This finding suggests that vermicomposting not only supports sustainable agriculture but may also enhance the therapeutic profile of edible fungi. While the study design provided robust evidence of haematological modulation, it did not account for sex-specific responses or pre-intervention baseline data—factors which may influence interpretative granularity. Additionally, the physiological status of the rats was not standardized, which could introduce biological variability.

In conclusion, the haematological improvements observed in rats consuming *P. ostreatus* grown on specific animal waste-enriched substrates affirm the nutritional and potential medicinal value of such dietary interventions. These findings support the strategic use of mushroom-based diets in functional feed development and invite further exploration into the molecular mechanisms underpinning these effects.

Conclusion
This study establishes that the haematological profile of albino rats is significantly influenced by dietary inclusion of *Pleurotus ostreatus*, particularly when cultivated on nutrient-dense substrates derived from animal manures. The findings highlight a substrate-dependent enhancement in key haematological indices—namely red blood cell count, haemoglobin concentration, packed cell volume, and white blood cell counts—suggesting improved erythropoietic and immunological responses. Among the substrates evaluated, poultry and goat dung-supported mushroom growth yielded the most pronounced physiological benefits. The differential performance observed between compost and vermicompost-based diets further underscores the value of bioconversion processes in augmenting nutrient bioavailability. Vermicomposting, through microbial and enzymatic action, appears to enhance the therapeutic potential of edible mushrooms, possibly by enriching them with hematopoietically active micronutrients and immune-modulatory compounds (Edwards et al., 2004; Wasser, 2011).

These insights not only reaffirm the functional role of *P. ostreatus* as a dietary adjunct but also point to the critical influence of cultivation substrates in determining its biomedical efficacy. For medical microbiology and nutritional science, such data provide a compelling basis for the development of substrate-optimized functional foods. Future studies should extend these findings by exploring the molecular mechanisms underlying these haematological effects and their translational relevance to human health.

Recommendations
1. In light of the findings, it is recommended that *Pleurotus ostreatus* cultivated on enriched organic substrates—particularly poultry and goat dung—be explored further as a functional dietary component with haematological benefits. Its potential use in both animal feed and human nutrition warrants attention, especially in regions facing protein malnutrition or micronutrient deficiencies.
2. Given the observed substrate-specific variations in haematological outcomes, future cultivation of medicinal mushrooms should not be approached generically. Instead, targeted substrate optimization should be prioritized to enhance bioactive compound synthesis. This could be advanced through controlled agronomic studies focusing on the biochemical transformation of specific manure types during composting and vermicomposting (Gbolagade et al., 2006; Edwards et al., 2004).
3. Moreover, clinical studies are strongly advised to validate these preclinical outcomes in human populations, with particular interest in anaemic individuals, immunocompromised patients, and those requiring nutritional rehabilitation. Molecular investigations into the expression of erythropoiesis-related genes and immune biomarkers following mushroom intake may yield insights into mechanistic pathways (Wasser, 2011; Zhang et al., 2007).
4. From a public health standpoint, integrating substrate-enriched mushrooms into community nutrition programs could offer an affordable and sustainable approach to improving haematological health. Policymakers should also consider supporting rural mushroom farming initiatives that utilize agricultural waste streams, promoting both health and environmental sustainability.

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