HAEMATOLOGICAL PARAMETERS OF RATS FED MUSHROOM, PLEUROTUS SAJOR-CAJU DIETS AND OROGASTRICALLY DOSED WITH PROBIOTIC LACTOBACILLUS FERMENTUM OVL

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ABSTRACT: In this study, the effect of edible mushroom, Pleurotus sajor-caju and oral administration of probiotic, Lactobacillus fermentum OVL on the haematological parameters of wistar albino rats were examined. There were no significant differences (Pe" 0.05) in the packed cell volume (PCV), haemoglobin (Hb) and white blood cell (WBC) count of rats placed on the above diet when compared to the control. There were however, significant differences (Pd"0.05) in the lymphocytes, neutrophil and eosinophil counts of rats. The neutrophil count of rats fed mushroom diet and dosed with Lactobacillus fermentum OVL increased while the lymphocyte count decreased. The lymphocyte count (55.67%) was however high in rats placed on casein diet and dosed with Lactobacillus fermentum OVL. This observation suggest that there was no potential synergy in the elevation of lymphocyte counts of rats fed mushroom diet and orogastrically dosed with Lactobacillus fermentum OVL, while there was sign of synergistic effect in the elevation of neutrophilic cells in rats fed mushroom diet and orogastrically dosed with Lactobacillus fermentum OVL. Neutrophils play a major role in innate immunity.

KEY WORDS: Haematology, Lactobacillus fermentum OVL, Mushroom, Rats

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INTRODUCTION

The maintenance of intestinal microbial balance had been found to promote good health in man and in animals (Fuller, 1989, FAO/WHO, 2001). Live microbial food supplements (probiotics) mainly from the lactic acid bacteria (LAB) group had been reported to be useful in bringing about intestinal microbial balance (Fuller, 1989). Several health promoting benefits such as immunostimulation, prevention of gastrointestinal tract (GIT) infections, growth enhancement of farm animals, anticholesterolaemic effects had been reported when probiotics are administered to man and animals (FAO/WHO, 2001).

The level of probiotic organisms in the GIT can be increased through the use of prebiotics (Crittenden et al., 2001). Prebiotics are food ingredients that are largely undegraded in the small bowel and can beneficially affect the host by selectively stimulating the growth and activity of one or limited number of bacteria (Gibson and Roberfroid, 1995). The consumption of non-digestible ingredients had been found to promote the growth of probiotic organisms especially bifidobacteria (Van Loo et al., 1999).

Mushrooms are food substances that are rich in non-digestible dietary fibres belonging to the β-glucan, chitin, and heteropolysaccharides, making up to as much as 10 – 50% of dry matter (Mizuno, 1999). The presence of high non-digestible material in mushroom may encourage the growth of probiotics in the GIT. Earlier reports of Oyetayo and Oyetayo (2005) showed that faeces of rats fed mushroom, P. sajor-caju had a higher lactobacilli count and a concomitant decrease in the count of harmful population, such as E. coli as compared to the control group fed protein diet that had a significantly higher E. coli count. The fibre content of edible mushroom, P. sajor-caju, was 17.07% (Oyetayo and Oyetayo, 2005) and this may be responsible for the increase in beneficial bacteria (lactobacilli) found in the faeces of rats fed diet compounded from P. sajor-caju. It has been reported that dietary fibre promotes the growth of fermentative species such as lactobacillus (Walker and Duffy, 1998).

Haematological studies had been found to be useful tools in diagnosing state of health (Cheesbrough, 1991) as the effect of food consumed can be monitored using these parameters. Blood cells are also useful tools in monitoring immunological status of subjects (Baker and Silverton, 1985). There is dearth of
information on the effect of edible mushroom, *P. sajor-caju* and oral administration of probiotic on the haematological parameters of experimental rats. The present study was therefore aimed at the evaluation of the haematological profile of rats fed edible mushroom, *P. sajor-caju* diet and oral administration of the probiotic, *Lactobacillus fermentum* OVL.

**MATERIALS AND METHODS**

*Lactobacillus Culture*

*Lactobacillus fermentum* OVL was isolated from Kunnu, a locally fermented alcoholic beverage brewed from sorghum. The isolate was characterised using colonial, morphological, and biochemical properties (Parker and Collier, 1990). Preliminary screening have shown that the isolate had probiotic properties such as: survival at acidic pH, resistance to common antibiotics and the inhibition of growth of pathogens and food spoilage bacteria.

The isolate was cultured in deMann Rogosa and Sharpe (MRS) broth (LAB M) and incubated at 37°C for 2 days to obtain large cell concentration. The cells were lyophilised using the method described by Fujiwara et al (2001). The concentration of the cell, which was 5.9x10^{13} cfu/g, was determined by serial dilution techniques.

**Source of Plerotus sajor-caju**

Fresh fruitbodies of edible mushroom, *P. sajor-caju*, collected from forest by local farmers were bought at the Oba’s Market, Akure. The mushroom samples were oven dried at 60°C and powdered using a Philip blender.

| TABLE 1. Per Cent Composition of Experimental Diets |
|-------------------------------|--------|--------|
| Ingredients                  | CD     | MD     |
| Corn Starch                  | 80     | 80     |
| Casein                       | 10     | -      |
| Mushroom protein*            | -      | 10     |
| Vegetable oil                | 5      | 5      |
| Vitamin/mineral premix**     | 5      | 5      |

CD: Casein diet, MD: Mushroom diet

* *Mushroom (49.89g) was added to diet (MD) to make 10% Mushroom protein in the compounded diet.

**Composition of vitamin/mineral premix (A Product of Green Field Nigeria Limited, Lagos): Vit. A (6,000,000 iu); Vit D₃ (1,300,000 iu); Vit E (3,000 iu); Vit K₃ (2000mg/kg); Riboflavin B₂ (4,000mg/kg); Pyridoxine B₆ (1,500mg/kg); Thiamine B₁ (2,000mg/kg); Vit B₁₂ (10mg/kg); Niacin (15,000mg/kg); Pantothenic acid (5,000mg/kg); Folic acid (20mg/kg); Biotin (20mg/kg); Manganese (80g/kg); Zinc (50g/kg); Iron (20g/kg); Copper (5g/kg); Iodine (1.2g/kg); Selenium (200mg/kg); Cobalt (200mg/kg).

**In vivo Feeding Trial**

Sixteen (16) albino rats (*Rattus norvegicus*) aged 4 – 5 weeks obtained from a research farm in Ile-Ife, Nigeria were acclimatised for one week on grower’s mash purchased from Bendel Feed, Nigeria. The rats weighed 58 – 82g after the acclimatisation. The rats were divided into groups of 4 rats each. The control (diet CD) was compounded from casein while diet MD was compounded from mushroom, *P. sajor-caju* as shown on Table 1. Treatments CDL and MDL in addition to being fed on diet CD and MD respectively were also dosed with 0.3ml of 5.9x10^{13} cfu/g of *Lactobacillus fermentum* OVL every 7 days. The rats were placed on the diets above for 28 days.

**Blood Analysis**

Blood samples were collected from rats by cardiac puncture over EDTA. Standard methods described by Cheesbrough (1991) was used to determine the following blood parameters: packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), mean cell haemoglobin concentration (MCHC) and differential leucocyte count for lymphocytes, neutrophil, eosinophil, basophil, and monocytes.

**Data Analysis**

Data gathered from the various analyses were processed using one-way analysis of variance (ANOVA) and the means were compared using Duncan Multiple range test.

**RESULTS**

The PCV, haemoglobin and WBC of rats fed diets compounded from mushroom and the casein diet (control) were not significantly different (P< 0.05) (Table 2). The differential leucocyte count is presented on Table 3. Neutrophilic cells were higher in rats fed mushroom diet and orogastrically dosed with *Lactobacillus fermentum* OVL (MDL) and casein diet (CD). The lymphocytic cells were on the other hand higher in rats fed mushroom diet (MD). Rats fed casein diet and orogastrically dosed with *Lactobacillus fermentum* OVL (CDL) also had a high lymphocyte count. The lymphocyte count (41.67%) was however lower in rats fed diet compounded from *P. sajor-caju* and orogastrically dosed with *Lactobacillus fermentum* OVL (MDL) when compared to rats fed mushroom diet (MD) with lymphocyte counts of 63.33%.

A significant difference in the eosinophil cells (8.33%) in rats fed mushroom diet (MD) alone and rats fed *P. sajor-caju* and orogastrically dosed with *Lactobacillus fermentum* OVL (MDL) that has 6.00% eosinophil counts.

**DISCUSSION**

Probiotics are known to confer health benefits such as, immunostimulatory, anticarcinogenic, anticholestorelaemic, growth enhancement and prevention of infection on the host (FAO/WHO, 2001). Similarly, mushrooms are known to possess health-promoting properties such as anticarcinogenic, immunostimulatory, hypocholesterolaemic
and antibacterial (Bahl, 1988, Mizuno, 1999, Wasser, 2002). They are known to be rich in non-digestible fibre, which encouraged the growth of probiotics (Crittenden et al., 2001). In a study, Oyetayo and Oyetayo (2005) reported that rats fed diet compounded from mushrooms, P. sajor-caju, effectively promoted the growth of probiotic lactobacilli. Non-digestible fibres that encourage the growth of beneficial microorganisms in the GIT had been termed prebiotics. The concept of synbiotic fibres that encourage the growth of beneficial microorganisms promoted the growth of probiotic lactobacilli. Non-digestible diet compounded from mushrooms, P. sajor-caju, are known to be rich in non-digestible fibre, which are known to express receptors that specifically recognise microorganisms and efficiently ingest and destroy these pathogens (Ishikawa and Miyazaki, 2005).

TABLE 2. Haematological Profile of Rats Fed Probiotic, Lactobacillus fermentum OVL and Edible Mushroom, Plerotus sajor-caju

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PCV(%)</th>
<th>Hb (g/dl)</th>
<th>WBC (mm³)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>43.00±1.53</td>
<td>14.00±1.00</td>
<td>5.00±0.12</td>
<td>0.33±0.00</td>
</tr>
<tr>
<td>MLD</td>
<td>41.33±1.33</td>
<td>13.00±1.00</td>
<td>4.40±0.40</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>CD</td>
<td>46.00±1.15</td>
<td>15.00±1.00</td>
<td>4.13±0.24</td>
<td>0.33±0.01</td>
</tr>
<tr>
<td>CDL</td>
<td>43.33±1.76</td>
<td>14.00±1.00</td>
<td>4.63±0.82</td>
<td>0.33±0.01</td>
</tr>
</tbody>
</table>

CD: Casein diet, MD: Mushroom diet, MLD: Mushroom diet + Lactobacillus fermentum OVL, CDL: Casein diet + Lactobacillus fermentum OVL, Trt: Treatment, PCV: Packed cell volume, Hb: Haemoglobin, WBC: White blood cell, MCHC: mean cell haemoglobin concentration. Values are means ± Std error of 4 rats per treatment. Values with different superscript are significantly different (Pd<0.05).

TABLE 3. Differential Leucocyte Counts of Rats Fed Probiotic, Lactobacillus fermentum OVL and Edible Mushroom, Plerotus sajor-caju

<table>
<thead>
<tr>
<th>Trt</th>
<th>Neutrophil (%)</th>
<th>Eosinophil (%)</th>
<th>Lymphocyte (%)</th>
<th>Monocyte (%)</th>
<th>Basophil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>26.67±2.72</td>
<td>8.33±4.17</td>
<td>43.33±4.16</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>MLD</td>
<td>59.00±3.61</td>
<td>6.00±1.15</td>
<td>41.67±1.06</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>CD</td>
<td>60.67±2.03</td>
<td>3.00±1.67</td>
<td>35.00±1.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>CDL</td>
<td>38.00±4.10</td>
<td>6.00±2.08</td>
<td>55.60±4.04</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

CD: Casein diet, MD: Mushroom diet, MLD: Mushroom diet + Lactobacillus fermentum OVL, CDL: Casein diet + Lactobacillus fermentum OVL, Trt: Treatment. Values are means ± std error of 4 rats per treatment. Values with different superscript are significantly different (Pd<0.05).

had been proposed to characterise colonic food with prebiotic and probiotic properties as health enhancing foods (Gibsons and Roberfroid, 1995).

In the foregoing, haematological parameters of rats fed prebiotic edible mushroom, P. sajor-caju, and orogastrically dosed with probiotic, Lactobacillus fermentum OVL showed no significant difference (Pd>0.05) in the PCV, WBC and haemoglobin of rats fed mushroom diet (MD) when compared with rats fed casein diet (CD) (Table 2). In a study, Anukam et al. (2004) had earlier reported that feeding Sprague-Dawley rats with probiotic, Lactobacillus fermentum RC-14, had no significant effect on the blood parameters such as WBC, PCV and haemoglobin. There were however significant differences (Pd<0.05) in some of the differential leucocyte cells. The neutrophil and lymphocyte counts of the rats in the various treatment groups show significant differences (Pd<0.05) between the treatment groups (Table 3). The neutrophil cells were higher in the rats fed mushroom diet and orogastrically dosed with (MDL) and casein diet (CD). Neutrophil count was however lowest in the rats fed mushroom diet alone. The major observation was a sign of potential synergy in the production of neutrophil (59.00%) in the rats fed mushroom diet and dosed with Lactobacillus fermentum OVL that was significantly higher than the neutrophil count (26.68%) of rats fed mushroom diet (MD) alone. Neutrophils are known to express receptors that specifically recognise microorganisms and efficiently ingest and destroy these pathogens (Ishikawa and Miyazaki, 2005).

Lymphocyte counts (63.33%) was higher and significantly different (Pd<0.05) in rats fed mushroom diet (MD) when compared to the other treatment groups. Lymphocytes are primarily responsible for humoral antibody formation and cellular immunity (Schalm et al., 1975). Mushrooms had been known to possess immunostimulatory property (Mizuno, 1999). There was no sign of potential synergy in the elevation of lymphocytes in the blood of rats fed mushroom, P. sajor-caju and dosed with Lactobacillus fermentum OVL.

Eosinophils counts (8.33%) were higher in rats fed mushroom diet (MD). Eosinophils are primarily assigned the function of detoxification and phagocytosis, however, their phagocytic capacity is limited (Clime et al., 1968).

Based on the haematological parameters monitored, the results obtained from this study suggest that there was no sign of toxicological effect on rats fed edible mushroom, P. sajor-caju and orogastrically dosed with probiotic, Lactobacillus fermentum OVL. There was no potential synergy in the elevation of lymphocyte counts of rats fed mushroom diet and dosed with Lactobacillus fermentum OVL (MDL), however, there was increase in neutrophil counts of rats fed MDL diet. Hence, a combination of mushroom diet, P. sajor-caju and the probiotic, L. fermentum OVL may encourage elevation of neutrophilic cells which plays the major role in innate immunity.

REFERENCES


Probiotics and haematological parameters


