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Effect of Different Concentrations of *Bacillus cereus* and its Bacteriocin on Some Hematological Indices of Wistar rats

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Abstract: *Bacillus* is an interesting genus to investigate since it produces a diverse array of antimicrobial peptides representing several different basic chemical structures. The production of bacteriocins or bacteriocin-like substance has already been described for *B. substilis*, *B. stearothermophilus* and other *Bacillus* sp. The present study assessed the effects of *Bacillus cereus* and its bacteriocin at different concentrations on the hematological parameters of male and female Wistar rats. For the acute intramuscular toxicity test, 10 Wister rats were randomly divided into five groups of two each. Each group constitutes a male and a female rat. The first and second group received *Bacillus cereus* at doses equivalent to 10^2 and 10^4 CFU while the third and fourth groups received the bacteriocin of the bacilli organism at the same concentration. The fifth group was used as the control and was given distill water. Blood samples were collected for hematology at day 0, 2, 10 and 15. The body weights before and after the experimental period were also noted. Analysis of variance (ANOVA) was used to determine the degree of significance between the baseline and subsequent days at $p<0.05$. There were no pathologic significant hematologic changes in the treated group. However, it was observed that lower concentration of *Bacillus cereus* and its bacteriocin is preferred to higher concentration of the Bacilli organism and its bacteriocin.

Key words: *Bacillus cereus*, bacteriocin, hematological parameters, rats, toxicity

INTRODUCTION

Bacillus cereus is one of around 60 representatives of the widely varied Bacillus genus. Along with the very similar species *B. mycoides, B. thuringensis and B. anthracis.* It comprises the so called "*Bacillus cereus* group". The differences between these four species are very small. *B. cereus* is found frequently as a saprophyte in soil, water, vegetation and air, from where it is easily transferred to food, either from the original raw material or during the food poisoning. It is common in dried foodstuffs, spices, cereals, meat, eggs, milk and milk products, cooked and inappropriately kept food (Kramer and Gilbert, 1989; Becker *et al*., 1994; Notermans *et al*., 1997). The colonization of different ecological niches is enabled by its extremely good adaptability and resistance to various influences. *B.cereus* produces endospores that survive pasteurization and are also resistant to various disinfectants. It also forms enzymes such as lipases, proteases, xylanases and others. In milk and milk products, it decomposes casein into peptides and amino acids, and milk fat into free fatty acids, thus degrading the quality of milk products and shortening their shelf life. *B.cereus* produces different types of toxins, hemolysins and phospholipases (Griffiths, 1990). Three types of diarrheal enterotoxins have been discovered so far, with the most research done on hemolytic (HBL) and Non-hemolytic Diarrheal Enteretoxin (NHE).The emetic syndrome is a consequence of emetic toxin formation in food (Granum *et al*., 1993; Dufrenne *et al*., 1995; Beecher and Wong, 1997). *B. cereus* and some closely related species from the genus *Bacillus* have several features including the production of various biologically active metabolites i.e. antibiotics, proteinases and bacteriocins that make them attractive candidates for biological control agents. It is well known that most, If not all bacterial species are capable of producing a heterogeneous array of molecules in the course of their growth in vitro (and presumably also in their natural habitats) that may be inhibitory to other bacteria (Tagg, 1976).

In the production of food, it is crucial to take proper measures for ensuring its safety and stability during the shelf life. Food preservation is carried out to maintain the quality of raw material and physicochemical properties as well as functional quality of the product whilst providing safe and stable product. Preservation aims at either eliminate or reducing the outgrowth potential of spoilage and pathogenic organisms in foods as well as consumer interest for high quality products with improved organoloptic and nutritional quality while maintaining microbial safety. In general, biological preservation approaches seem attractive as a safety parameter in foods

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with reduced contents of ingredients such as salt, sugar, fat and acid that usually serve as factors potentially inhibitory to microbial growth. It is expected that biological preservation method may enjoy better consumer acceptance than their preservation counterparts that use traditional chemical preservatives.

Bacteriocins are compounds produced by bacteria that have biologically active protein moiety and a bactericidal or bacteriostatic action Line *et al*. (2008). Bacteriocin from Gram-positive organism such as Lactic Acid Bacteria (LAB), have attracted much attention and have been the subject of intensive investigation due to their extensive incorporation as biopreservatives ingredients into model foods particularly in the dairy industry Diop *et al*., (2007) and also in human therapeutics Martin-Visscher *et al*. (2008).

The aim of this study was to investigate if dose range has any significance in determining the effectiveness of *Bacillus cereus* and its bacteriocin as food preservative.

MATERIALS AND METHODS

This study was carried out in the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria between the month of January to December, 2010.

Bacterial strain: *Bacillus cereus* was harvested from yam floor and identified on the basis of its cultural, physiological, morphological and biochemical characteristics (Barrow and Feltham, 1993). The isolate was examined for bacteriocin production via inhibition zone using the Agar Well Diffusion (AWD) assay Lasta *et al.* (2008) and then stored in glycerol at temperature of -20ºC

Test animals: Male and female albino rats, *Rattus norvegicus albinus* weighing 80-120 g were purchased from the animal house, University of Ibadan, Nigeria. Rats were kept under the laboratory conditions of 25±5ºC and 65±5% R.H., three weeks as an acclimatization period. They were housed in metal cages (25×20×15 cm) and maintained on *ad libitum* diet and water. This diet contained all the dietary needs and was obtained from Mokola Market, Ibadan, Oyo State, Nigeria. Animal experiments and housing procedures were performed in accordance to the animal care rules and they were approved by the authorities of the University.

Preparing of inoculums: *Bacillus cereus* from yam floor was stored in glycerol at -20ºC and was purified by sub culturing of the colonies in a nutrient agar plate and incubated at 30-37ºC for 24 h in an inverted position. In effort to obtain pure strain, a microbial colony growing separately from other colonies was picked with the aid of a sterile wire loop. This was streaked out on a new nutrient agar plate and incubated at 37ºC. The sub culturing was done several times to obtain a pure colony. Serial dilution of the Bacilli organism was done to determine the concentration of the organism and its bacterocin to be inoculated into the rats. 0.1 mL of the sample organism diluted to 10^{-10} was inoculated into a Petri dish containing prepared nutrient agar and incubated at 30-37ºC for 24 h. The total colony forming for *Bacillus cereus* at 10^{-10} serial dilution was 2.52×10^{13} Cfu/mL.

Screening for bacteriocin: *Bacillus cereus* was placed in wells (5 mm in diameter) cut in nutrient agar plates (2 cm) seeded with the Gram negative *micococus luteus* organism.

Harvesting of bacteriocin: *Bacillus cereus* which showed antimicrobial activity against indicator organism by zones of clearance on nutrient agar plate were grown in 10 mL of nutrient broth and incubated at a temperature of 37ºC for 18-24 h. The broth was centrifuged at 3500 revolutions for 15-20 min after which the bacteriocin was drawn out into another test tube using a pipette to prevent mixing with the bacteria cells below the test tubes. Then it was decanted into sterile test tubes, adjusted to pH 6.5-7.0 with NaOH (40G/1000 mL) to remove organic acid effect. H_2O_2 was neutralized by addition of catalase from bovine liver at 200 μ/mL. The mixture of the bacteriocin of culture, NaOH and catalase was filtered and sterilized with a 0.2 μm Millipore filter membrane. This filtrate (bacteriocin) was covered with foil paper and then stored at 4ºC to prevent contamination until use.

Experimental design: Ten rats were divided randomly to five groups. Each group having a male and female representative. The first and second groups were given 10^2 and 10^4 CFU doses of *Bacillus cereus* which are equivalent to 0.1 and 0.3 mL of 1 mL broth culture respectively (data of serial dilution and calculation not shown). While the third and fourth groups were given 0.1 and 0.3 mL does of bacteriocins from 10^2 and 10^4 CFU, respectively. Both administrations were done intramuscularly using the thigh muscles. The fifth group received distilled water and served as the control. At day 0, before inoculation of samples viz *Bacillus cereus* and its bacteriocin, the rats were weighed and blood samples were collected for a baseline data. At day 2, 10 and 15 post inoculums, blood samples were collected and the rats were re-weighed on the 15th day.

Blood sample: Blood samples (pre and post innoculum) were individually collected from each rat. During each

collection, 1 mL of blood sample was collected from each rat in replicate from the eye by inserting a capillary tube into the media canthus of the eye and blood was drawn through the capillary tube into the ependoff tube containing EDTA as anticoagulant (50 mL/mL) for hematological analysis.

Hematological examination: The hemoglobin concentration was done as described by Schalm *et al*. (1975) using the cyanomethaemoglobin method. Packed Cell Volume (PCV) was done by conventional method offilling the capillary tube with blood as described by Schalm *et al*. (1975) and read with a microhaematacrit reader. Erythrocyte count was determined by the haemocytometer method as described by (Coles, 1986). The leucocytes and leucocytes differential counts were also determined. Erythrocyte indices were from value obtained from red blood cell count, hemoglobin concentration and packed cell volume.

Statistical analysis: Analysis of variance (ANOVA) (SPSS, 2006) was used to determine significant difference between baseline and subsequent days at $p<0.05$.

RESULTS

The mean inhibition zones of *Bacillus cereus* using Agar Well Diffusion method was 15 mm. No significant changes in body weights were found in rats in each group (Table 1).

Clinical findings:Five to seven days post administration, some rats showed signs of alopecia Viz: female rats dosed with *Bacillus cereus* at 10^2 CFU, female rats given bacteriocin of *Bacillus cereus* at 10^4 CFU and female rats used as control. After the collection of blood samples at day 2 and 10, male rats administered bacteriocin of *Bacillus cereus* and *Bacillus cereus* respectively at 10^4 CFU were found dead.

Effects on hematological parameters: Female rats dosed with *B. cereus* at 10^2 CFU showed a significant

Table 1: Weights of rat before and after inoculation of *Bacillus cereus* (B) and its bacteriocin (b)

	FOB^2	MOB^2	FOB^4	MOB^4	FbB^2	MbB^2	FbB^4	MbB^4	FC
Before	120	80	80	80		80	80	80	12080
After	123	81	80	-	80	82	80	-	80

FOB^2: female rats given *Bacillus* organism at 10^2 CFU; MOB^2: male rats given *Bacillus* organism at 10^2 CFU; FOB^4: female rats given *Bacillus* organism at 10^4CFU; MOB^4: male rats given *Bacillus* organism at 10^4CFU; FbB^2: female rats given bacteriocin at 10^2 CFU; MbB^2: male rats given bacteriocin at 10^2 CFU; FbB^4: female rats given bacteriocin at 10^4CFU; MbB^4: male rats given bacteriocin at 10^4CFU; FC: female rats used as control; MC: male rats used as control

Table 2: Hematological data of female rats before (Baseline) and after *B. cereus* application

Organism	Type	Dosage		PCV	Hb (g/dL)	RBC ($\times10^4/\mu L$)	WBC ($\times10^4/\mu L$)	PLT ($\times10^4/\mu L$)
Female	B	10^2	Baseline	40.50±0.50	13.35±0.15	15.31±2.91	22.20±4.20	12.00±0.00
			Day2	30.50±0.50	10.05±0.15	6.50±0.60	9.90±0.30	11.00±1.00
			Day 10	49.00±3.00	16.20±1.00	27.63±1.21	23.00±2.40	11.00±1.00
			Day 15	32.00±0.00*	12.50±2.00	15.00±0.04	9.80±0.20	13.00±1.00
		10^4	Baseline	38.50±1.50	12.70±0.50	8.94±1.30	17.95±2.05	14.00±4.00
			Day 2	30.50±0.50	10.05±0.15	9.63±2.8	1 14.10±4.50	12.00±2.00
			Day 10	41.00±0.00	13.50±0.00	20.09±0.01	11.10±0.10	10.50±0.50
			Day 15	35.00±5.00	11.55±1.65	17.06±1.02	8.80±0.20	12.00±0.00
	Control	Control	Baseline	37.00±1.00	12.30±0.40	11.70±2.50	24.60±6.00	12.00±2.00
			Day 2	24.00±1.00	7.85±0.35	4.38±0.26	6.40±0.00	11.00±1.00
			Day 10	44.00±0.00	14.70±0.00	22.86±0.00	12.40±0.00	18.00±0.00
			Day 15	41.00±2.00	13.55±0.65	15.02±0.58	16.20±1.40	10.50±1.50

Organism	Type	Dosage		MCV	MCH	LYM	NEUT
Female	B	10^2	Baseline	27.00±6.00	8.50±1.50	76.00±0.00	23.50±0.50
			Day 2	46.50±3.50	15.00±1.00	41.50±1.50*	58.50±1.50*
			Day 10	17.50±0.50	58.00±1.00*	72.00±2.00 2	8.00±2.00
			Day 15	21.00±0.00	69.50±0.50*	65.00±0.00	35.00±0.00*
		10^4	Baseline	43.50±4.50	13.50±1.50	70.00±0.00	30.00±0.00
			Day 2	25.50±0.50	11.50±3.50	23.50±3.50*	76.00±4.00
			Day 10	20.00±0.00	67.00±0.00*	55.50±0.50	44.00±0.00
			Day 15	20.00±2.00	67.00±6.00	57.50±2.50*	42.50±2.50
	Control	Control	Baseline	32.50±6.50	10.00±2.00	62.00±3.00	38.00±3.00
			Day 2	54.50±5.50*	17.50±1.50*	32.50±4.50*	67.00±5.00*
			Day 10	19.00±0.00	64.00±0.00	60.00±0.00	40.00±0.00
			Day 15	27.00±2.00	95.00±3.00*	63.00±7.00	37.00±7.00

*: Mean is significantly different from baseline value at $p<0.05$; *: Each value represents mean±SD; PCV: Packed cell volume; Hb: hemoglobin; RBC: red blood cell count; WBC: White blood cells; PLT: Platelet count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; LYM: lymphocyte; NEUT: neutrophil; B: *Bacillus cereus*; 10^2: 10^2CFU; 10^4: 10^4CFU

Table 3: Hematological data of female rats before (Baseline) and after bacteriocins of *B. cereus* application

Type	Dosage		PCV	Hb (g/dL)	RBC (×10^4/μL)	WBC (×10^4/μL)	PLT (×10^4/μL)
B	10^2	Baseline	30.50±3.50	10.05±1.15	10.24±0.56	10.70±1.50	11.00±1.00
		Day 2	17.50±0.50	5.70±0.20	6.35±2.87	9.35±1.45*	10.50±0.50
		Day 10	41.50±0.50	13.80±0.10	25.83±4.03	13.11±1.10	11.00±1.00
		Day 15	36.50±0.50	12.05±0.15	15.69±0.29*	9.40±0.20	7.00±1.00
	10^4	Baseline	36.00±0.71	12.00±0.24	13.23±1.75	22.43±3.54	9.00±1.29
		Day 2	25.25±1.31	8.28±0.45*	5.18±0.26*	7.75±1.02*	12.50±2.22
		Day 10	38.75±2.93	12.83±0.99	26.55±2.25*	14.58±2.01	10.50±0.96
		Day 15	38.00±0.58	12.55±0.20	17.41±0.49	14.53±1.34	12.25±1.03
Control	Control	Baseline	36.50±2.50	12.05±0.85	11.04±1.36	21.20±9.40	9.00±0.00
		Day 2	27.00±0.00	8.90±0.00	5.16±0.00	9.30±0.10	19.00±1.00
		Day 10	46.00±0.00	15.20±0.00	25.24±0.00	18.80±0.00	15.00±0.00
		Day 15	36.00±0.00	10.40±0.00	18.10±0.00	12.00±0.00	8.00±0.00
Type	Dosage		MCV	MCH	LYM	NEUT	
B	10^2	Baseline	30.00±5.00	9.50±1.50	71.50±0.50	28.50±0.50	
		Day 2	34.50±15.50	12.00±6.00	23.50±1.50*	76.00±2.00*	
		Day 10	16.00±3.00	54.00±9.00	71.00±1.00	29.00±1.00	
		Day 15	23.00±0.00	76.50±0.50*	73.00±1.00	27.00±1.00	
	10^4	Baseline	35.50±2.33	11.00±1.08	67.50±1.44	32.50±1.44	
		Day 2	48.25±0.95*	15.50±0.50*	25.25±2.06*	74.25±2.17*	
		Day 10	14.25±1.38*	48.50±4.65*	65.00±4.65	34.50±4.63	
		Day 15	21.50±0.87*	72.00±3.24*	56.25±0.75*	42.75±0.48*	
Control	Control	Baseline	33.00±2.00	10.50±0.50	80.00±0.00	20.00±0.00	
		Day 2	52.00±0.00	17.00±0.00*	37.50±2.50*	62.50±2.50*	
		Day 10	18.00±0.00	60.00±0.00	76.00±0.00	24.00±0.00	
		Day 15	24.00±0.00	84.00±0.00	65.00±0.00	27.00±0.00	

*: Mean is significantly different from baseline value at p<0.05; *: Each value represents mean±SD; PCV: Packed cell volume; Hb: hemoglobin; RBC: red blood cell count; WBC: White blood cells; PLT: Platelet count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; LYM: lymphocyte; NEUT: neutrophil; B: *Bacillus cereus*; 10^2: 10^2CFU; 10^4: 10^4CFU

Table 4: Haematological data of male rats before (Baseline) and after *B. cereus* application

Organism	Type	Dosage		PCV	Hb (g/dL)	RBC (×10^4/μL)	WBC (×10^4/μL)	PLT (×10^4/μL)
	B	10^2	Baseline	34.50±0.50	11.45±0.25	8.74±0.96	17.10±1.30	12.00±2.00
			Day 2	19.00±1.00	6.20±0.30	10.67±1.47	13.40±1.60	10.00±2.00
			Day 10	41.50±0.50	13.70±0.20	22.07±0.29*	16.70±0.90	15.50±0.50
			Day 15	35.00±0.00	11.70±0.00	15.64±0.16	10.50±0.10	12.50±2.50
		10^4	Baseline	36.00±2.00	11.95±0.75	11.00±1.25	20.60±8.40	15.00±1.00
			Day 2	25.00±0.00	8.20±0.00	4.09±0.01	5.65±0.45	12.00±0.00
			Day 10	41.50±2.50	13.80±0.90	23.01±2.81	17.07±1.57	11.00±1.00
	Control	Control	Baseline	26.50±0.50	8.80±0.10	9.92±0.32	9.60±0.00	13.50±1.50
			Day 2	28.50±0.50	9.45±0.25	6.08±0.18*	7.40±2.60	13.00±2.00
			Day 10	41.00±0.00	13.70±0.00	20.76±0.00	18.20±0.00	20.00±0.00
			Day 15	38.50±2.50	12.80±0.90	17.24±0.36*	11.30±0.30	10.50±4.50
Organism	Type		Dosage	MCV	MCH	LYM	NEUT	
	B	10^2	Baseline	39.50±3.50	13.00±1.00	80.00±0.00	20.00±0.00	
			Day 2	17.50±1.50	5.50±0.50	33.00±2.00*	66.50±1.50*	
			Day 10	18.50±0.50	61.50±1.50*	84.00±6.00	21.00±1.00	
		10^4	Day 15	22.00±0.00	76.00±1.00*	60.50±0.50*	38.00±1.00*	
			Baseline	32.50±1.50	10.50±0.50	60.00±0.00	40.00±0.00	
			Day 2	61.00±1.00	20.00±0.00*	34.50±4.50	65.00±5.00	
			Day 10	18.00±1.00	59.50±3.50	61.00±1.00	38.50±0.50	
	Control	Control	Baseline	26.50±0.50	8.50±0.50	64.50±0.50	35.50±0.50	
			Day 2	46.50±0.50	15.00±0.00*	43.50±1.50	55.00±1.00*	
			Day 10	19.00±0.00	65.00±0.00	56.00±0.00	44.00±0.00	
			Day 15	22.00±1.00	73.50±3.50*	70.50±0.50	29.00±1.00	

*: Mean is significantly different from baseline value at p<0.05; *: Each value represents mean±SD; PCV: Packed cell volume; Hb: hemoglobin; RBC: red blood cell count; WBC: White blood cells; PLT: Platelet count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; LYM: lymphocyte; NEUT: neutrophil; B: *Bacillus cereus*; 10^2: 10^2CFU; 10^4: 10^4CFU

decrease (p<0.05) in PCV at day 15 as compared to the control group. There were no significant changes in Hb, RBC, WBC, PLT and MCV in the female rats treated with *B. cereus* at 10^2cfu, MCH showed a significant increase (p<0.05) at day 7 and 15, neutrophil decreased at day 2 and 15 while lymphocyte decreased at day 2. At 10^4 CFU, there were significant decreases in lymphocyte at day 2 and 15 (Table 2).

Female rats treated with the bacteriocin of *Bacillus cereus* at 10^4 CFU showed a significant decreases in Hb,

Table 5: Haematological data of male rats before (Baseline) and after bacteriocins of *B. cereus* application

Type	Dosage		PCV	Hb (g/dL)	RBC ($\times10^4/\mu L$)	WBC ($\times10^4/\mu L$)	PLT ($\times10^4/\mu L$)
B	10^2	Baseline	31.00±0.00	10.20±0.00	8.92±2.52	14.30±5.90	12.00±4.00
		Day 2	14.50±0.50*	4.70±0.20*	3.02±0.18	6.50±0.70	12.50±2.50
		Day 10	30.10±0.05	8.00±0.20	7.50±0.00	14.00±4.20	10.00±1.00
		Day 15	28.00±0.00	6.00±0.05	5.70±0.50	11.00±1.07	11.50±1.00
	10^4	Baseline	38.00±0.00	11.90±0.00	11.41±0.01	19.00±0.50	10.00±1.00
		Day 2	25.50±0.50	7.90±0.00	5.13±1.07	7.80±1.60*	11.00±2.00
Control	Control	Baseline	40.00±1.00	13.20±0.30	12.64±2.56	15.90±4.70	11.00±1.00
		Day 2	24.00±1.00	7.85±0.35*	4.34±0.26	6.20±1. 00	14.00±2.00
		Day 10	40.00±0.00	13.20±0.00	20.22±0.00	12.40±0.00	16.00±0.00
		Day 15	32.00±0.00	10.50±0.00	13.68±0.40	18.55±0.75	12.00±2.00
Type	Dosage		MCV	MCH	LYM	NEUT	
B	10^2	Baseline	37.50±10.50	11.50±3.50	60.00±0.00	40.00±0.00	
		Day 2	50.00±0.00	18.50±3.50	36.00±1.00*	63.50±1.50*	
		Day 10	32.50±1.00	65.50±1.50*	61.00±1.00	39.00±1.00	
		Day 15	35.00±0.057	1.00±0.05*	55.50±0.00	41.50±0.05	
	10^4	Baseline	30.00±0.00	10.00±0.00	60.50±0.50	39.50±0.50	
		Day 2	43.00±8.00	14.00±0.00	27.00±1.00*	68.00±4.00	
Control	Control	Baseline	32.50±7.50	10.50±2.50	72.50±2.50	27.00±3.00	
		Day 2	55.50±5.50	18.00±2.00*	22.00±2.00	77.50±2.50	
		Day 10	19.00±0.00	65.00±0.00	70.00±0.00	30.00±0.00	
		Day 15	23.00±1.00	76.50±2.50*	61.00±1.00	37.00±3.00	

*: Mean is significantly different from baseline value at $p<0.05$; *: Each value represents mean±SD; PCV: Packed cell volume; Hb: hemoglobin; RBC: red blood cell count; WBC: White blood cells; PLT: Platelet count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; LYM: lymphocyte; NEUT: neutrophil; B: *Bacillus cereus*; 10^2, 10^2CFU; 10^4, 10^4CFU

RBC, WBC at day 2 while at day 10, RBC increased ($p<0.05$). Rats treated with the bacteriocin of *B. cereus* at 10^4 CFU increased significantly at day 2 and decreased significantly at 15. At 10^2 cfu and 10^4 CFU, there were significant increase ($p<0.05$) in MCH at day 15. At 10^2 CFU and 10^4 CFU there was a significant decrease in lymphocyte at day 2 and 15. Neutrophil increased significantly, At day 2 and 15, female rats treated with the bacteriocin at 10^2 cfu and 10^4 cfu showed a significant increase in neutrophil while when dosed at 10^4 cfu, it decreased significantly at day 15 (Table 3).

Male rats treated with *B. cereus* at 10^2 and 10^4 CFU showed no significant change in PCV, Hb, WBC, PLT and MCV throughout the experiment. At day 10, RBC increased significantly when 10^2 CFU of *B. cereus* was given to rats and MCH increased significantly at day 10 and 15 when given the same dose while at 10^{-4} CFU, it increased significantly at day 2. Male rats treated at 10^2 CFU, showed significant decrease in lymphocyte and significant increase in neutrophil at day 2 and 15 (Table 4) Male rats dosed with the bacteriocin of *B. cereus* at 10^2 CFU showed a significant decrease in PCV, Hb and lymphocyte at day 2 and there was a significant increase in neutrophil at day 2 while MCH increased significantly at day 10 and 15. At 104CFU, PCV, WBC and LYM decreased significantly at day 2 (Table 5).

DISCUSSION

Alopecia which was observed in the female rats could be associated with gender and or an environmental condition in which the rats were allergic to. This may be true since the female control rat was observed to have loss of hair on the dorsal part of the body. The mortalities which were encountered during the experimental period could be due to an increase in the concentration of the Bacilli organism and its bacteriocin since such observation was not fond at lower doses. Increase in PCV, RBC and Hb is an indicator that the rats were not anemic, while decrease level is a sign of anemia. PCV measures the percentages by volume of packed RBC in the whole blood sample after centrifugation (Wynne and Edwards, 2003) while Hb test measures the amount of Hb in grams per dl of whole blood and provides an estimate of oxygen carrying capacity of the RBCs. From the result, a reduction of PCV, erythrocyte and consequent Hb concentration at day 2 may be due to the failure to supply the blood circulation with cells from haemohepatic tissues, since the liver has an important role in the regeneration of erythrocyte (Anubama *et al*., 2001). It may be due to reduction of blood during sampling or heamolysis of blood cells due to the intramuscular administration of *Bacillus cereus.* A significant increase in PCV, RBC and Hb by the bone marrow at day 10 is useful in increasing tissue oxygenation. WBC count is the number of WBC in a cubic millimeter of whole blood and is usually important in fighting against infections Schalm *et al*. (1975). The significant decrease in WBC from the hematological report indicates low level of infection in the experimental rats, or may be related to suppression of the production of the WBC resulting from reactions to substances Schroder *et al.* (2007). This can be an added advantage since increased numbers are associated with infections and leukemia's. Decrease in WBC count

normally reflects a decline in the production of defensive mechanism to combat infections. Such decrease will naturally make the rats more susceptible to various physiological stresses resulting in disease, poor growth and greater mortality. A significant increase in the WBC could be due to the presence of *B. cereus* in the circulatory system so that they could destroy and inhibit the activities of the substances in the system. The presence of lymphocyte in the circulatory system at day 10 may be due to their clearing activities of the cellular debris after the *Bacilli* organisms have been engulfed and destroyed by neutrophils. Mean Corpuscular Volume (MCV) measures the average volume of a red cell in an individual's blood. From the hematological result, the significant increase in MCV at day 2 shows that the anemia is regenerative. Therefore, MCV is normocytic. MCH (Mean Corpuscular Hemoglobin) which measures the weight of hemoglobin in a red cell of an individual sample increases significantly as the experiment progresses. This shows that the anemia is normochromic and tissue oxygenation is increased.

CONCLUSION

This study further accentuate the non-suitability of *B. cerus* or its bacteriocin as a food preservative. However, It can be deduced that product in which *Bacillus cereus* and its bacteriocin is being incorporated at lower concentration is preferred to higher concentration. Also, female consumers are at less risk to male consumers of products incorporated with the bacteriocin of *Bacillus cereus* at lower concentration.

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