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**Research Paper**

**Socio economic characteristics of fishermen in Jabel Awlia and El- Mawrada at Khartoum state, Sudan.**

Mohamed Ahmed F.A., Daldoum Daleel E. M, Idres Yagoub A.H, Abd-Al.Saeed Fadlalseed S., Mohammed Salh R.R., Ksrpos Kolia W.S., Mohammed A.A.

*Online J. Anim. Feed Res.*, 5(4): 95-100, 2015; pii: S222877011500016-5

**Abstract**

The present study aimed to provide baseline information of socio-economic status of Jabel Awlia and El-Mawrada fishery in the White Nile in Sudan due to there is lack of information in this field. Descriptive analysis was done for analyzing the raw data of the study by using Excel Microsoft Software 2003. Social data showed that male fishermen group was dominant in both sites (97.6%, 100%) respectively. Age groups of fishermen ranged between 20 to 70yrs; where age group 31- 40yr was dominant in Jebel Awlia and age group 41-50 was dominant in El-Mawrada. Six educational categories were recorded where primary education was dominant in the two location (48.8%, 44.4%) respectively; whereas, secondary education was the second in the two location (24.4%) and (27.8%) respectively. Part-time fishermen was dominant in both sites (34.1%, 11.1%); whereas full time fishermen was the lowest (4.9%, 16.7%). Most of fishermen were married (85%, 94%) in both sites; whereas ...[read more](#)

**Keywords:** Fishery, Fishermen, Economic, Nile, Fishing.

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**Research Paper**

**The performance of growing pigs fed diets containing different levels of sodium hydroxide-treated Sheanut kernel cake.**

Oddoye E.O.K., Nortey T. N., Owusu Ansah F and Gyedu-Akoto E.

*Online J. Anim. Feed Res.*, 5(4): 101-105, 2015; pii: S222877011500017-5

**Abstract**

The aim was to investigate the use of sodium hydroxide treated (0.01M) Sheanut kernel cake (TSNKC) as a feed ingredient in growing pig diets. The growth rate, feed intake and feed to gain ratio of growing pigs fed diets containing 100 (100USNKC/control), 100 (100TSNKC), 150 (150TSNKC) or 200 (200TSNKC) g kg<sup>-1</sup> of sodium hydroxide treated Sheanut kernel cake was investigated in two feeding trials set up as completely randomized designs with 4 treatments replicated 3 times and lasting 120 days. The two feeding trials were combined and analyzed as a split-plot, with the trials being the main plot and the experiment within the trial as the sub plot. Treatment 100TSNKC was significantly different ( $P < 0.05$ ) from treatment 200TSNKC. Treatments 100USNKC and 150TSNKC were not significantly different ( $P > 0.05$ ) from either 200TSNKC or 100TSNKC. Total feed intake for 200TSNKC was significantly lower ( $P < 0.05$ ) as compared to the other treatments but feed to gain ratio was not significantly different ( $P > 0.05$ ). It was concluded that it is possible to include up to 150 g kg<sup>-1</sup> sodium hydroxide treated Sheanut kernel cake in growing pig diets. Future work will need to look at ways of improving the palatability of sodium hydroxide treated Sheanut kernel cake and also look at carcass analysis and blood profiles.

**Keywords:** Sheanut, Sodium Hydroxide Treatment, Sheanut Kernel Cake, Growing Pigs, Feeding Trial.

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**Research Paper**

**The use of cassava root meal as a partial replacement for corn in diets for albino rats.**

Nortey T.N., Arkorful E., Sowah N. A., Naazie A.

*Online J. Anim. Feed Res.*, 5(4): 106-111, 2015; pii: S222877011500018-5

**Abstract**

The experiment was carried out to determine if partial replacement of maize with cassava root meal (CRM) in diets for albino rats will have an effect on performance, organ characteristics and blood parameters. Twenty five Sprague Dawley albino rats (F344 strain), initial body weight (216 ± 8g) were randomly assigned to five treatments (T1 to T5) in a completely randomized (CRD) arrangement. T1 was the control and contained zero CRM. T2 and T3 contained 30% CRM, while T4 and T5 contained 45% CRM. These levels of inclusion represented 50 and 75% replacement of corn in the diets respectively. T2 and T4 had 0.15% methionine (Met) while T3 and T5 had 0.3% Met. The rats were each fed a single diet for 28d. Average daily feed intake (ADFI) of rats on T1 was lower ( $P < 0.05$ ) than that for rats on T4 and T5 (12.12g vs.



12.77 and 12.64g) respectively. For diets with the same level of CRM, those with 0.3% Met had a lower consumption than those with 0.15% Met. There were no differences ( $P > 0.05$ ) in average daily gain (ADG) and feed conversion efficiency (FCE). Similarly there were no differences ( $P > 0.05$ ) in carcass, viscera and other internal organ weights. Results of this trial indicate that albino rats can tolerate diets with added CRM (45% of the diet) with no adverse effects on growth and internal organ characteristics. Future work will need to look at the possibility of using CRM at similar or higher levels in diets for growing pigs.

Keywords: Albino Rats, Cassava Root Meal, Performance, Carcass Characteristics, Blood Parameters

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## Research Paper

### Comparison between the percentage of incidence of mastitis caused by *Bacillus* spp and *Staphylococcus* spp in winter season in Khartoum state, Sudan.

Mohammed Salih R.R.

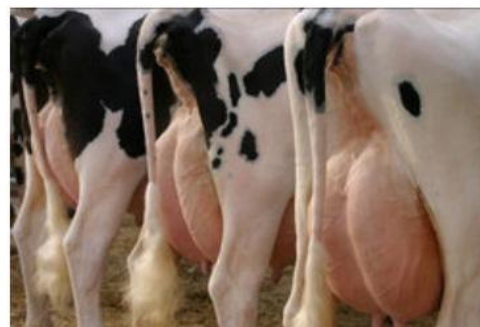
Online J. Anim. Feed Res., 5(4): 112-116, 2015; pii: S222877011500019-5

#### Abstract

This study was conducted in certain area at Khartoum State (Eltebna, Falasteen, Shambat, Hilat Kuku, Elhalfaia, Elsamrab and The University of Khartoum farms) in winter season to determine the type of mastitis and to compare between the incidence of mastitis caused by *Staphylococcus* spp and *Bacillus* spp. The total number of dairy cows, which were examined in 34 investigated farms, amounted to 500 animals, but the number of positive cows infected with mastitis were 100. The milk samples were collected from cows due to complain of owners from clinical cases of mastitis. Hundred milk samples were collected from apparent cases of mastitis. All mastitic cases were examined by visual examination and palpation of the udder: 55% acute mastitis, 44% chronic mastitis and 1% gangrenous mastitis were diagnosed. Milk samples were cultured in Blood agar and MacConkey's agar for 24 hours at 37°C. The isolation of *Bacillus* spp. amounted 74%, these constituted 31% *Bacillus coagulans*, 11% *B. cereus*, 9% *B. subtilis*, 9% *B. licheniformis*, 4% *B. circulans*, 2% *B. lentus*, 3% *B. mycooides*, 3% *B. amyloliquefaciens* and 2% *B. megaterium*. The percentages of acute mastitis caused by *B. coagulans* was 14%, *B. subtilis* 8%, *B. licheniformis* 7% and 2% for every followed *Bacillus* spp. (*B. cereus*, *B. circulans*, *B. lentus*, *B. mycooides*, *B. amyloliquefaciens* and *B. megaterium*). The percentage of chronic mastitis caused by *Bacillus* spp. were as follows: *B. coagulans* was 17%, *B. cereus* 9%, 2% for every *Bacillus* spp. (*B. licheniformis*, *B. circulans* and *B. lentus*) and 1% for every followed *Bacillus* spp. (*B. subtilis*, *B. mycooides*, *B. amyloliquefaciens* and *B. megaterium*). *Staph aureus* and *Staph hyicus* amounted to 24% and the percentage of chronic mastitis caused by *Staph aureus* was 44% and that caused by *Staph hyicus* was 8%. The percentage of acute mastitis caused by each species of *Staph* was the same 24%. Other bacteria were isolated from mastitic cows *Corynebacterium* spp. 1% and *Klebsiella* spp. 1% and the last one was isolated from gangrenous mastitis as first report in Sudan.

Keywords: Comparison, Incidence, Mastitis, *Bacillus* Spp., *Staphylococcus* Spp., Cattle, Winter, Khartoum, Sudan.

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## Research Paper

### Amelioration effect of probiotics on aflab1 induced hematological alterations in fresh water fish *Cyprinus carpio* L.

Pradeepkiran J.A., and Bhaskar M.

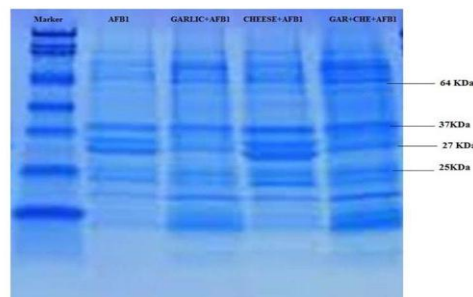
Online J. Anim. Feed Res., 5(4): 117-124, 2015; pii: S222877011500020-5

#### Abstract

The objective of this study was to examine protective effect of probiotics against aflatoxin B1 (AFB1) on hematological and serum parameters which include the RBC, WBC, albumins, globulins, serum creatinine, and ALT and AST of fresh water fish *Cyprinus carpio* L. The total hemobiochemical analysis was compared with the AFB1 induced and probiotics (garlic, cheese) treated groups. *Cyprinus carpio* L. (40 ± 10 g), were randomly divided into five experimental groups (15 fish per group). Group T1 represented the negative control fed with normal diet, and T2 was the positive control group fed with AFB1 contaminated diet. Groups T3, T4 were fed with AFB1-contaminated diet 200ppb supplemented with 2 mg/kg cheese, 2 mg/kg garlic, and Group T5 fed with AFB1-contaminated diet and 4 mg/kg bw (garlic + cheese) probiotic supplementation in 1:1 ratio respectively. Ingestion of AFB1-contaminated fish feed possess the adverse effects on hematological parameters like, total red blood cells numbers, relative number of lymphocytes, monocytes, neutrophils, basophils, and eosinophils in blood. Likewise AFB1 altered globulin, albumins, and total protein concentrations in serum fractions (GroupT2). Supplementation of probiotics cheese alone showed significant protective effect than garlic and in combination group (Group T5). Group 5 showed more or less similar to that of the control, in conclusion probiotics cheese and garlic showed significant combat effects in reducing hematological toxic effects of AFB1.

Keywords: Probiotics, Aflatoxin B1, Hematology, Cheese, Garlic.

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**ABSTRACT:** The present study aimed to provide baseline information of socio-economic status of Jabel Awlia and El-Mawrada fishery in the White Nile in Sudan due to there is lack of information in this field. Descriptive analysis was done for analyzing the raw data of the study by using Excel Microsoft Software 2003. Social data showed that male fishermen group was dominant in both sites (97.6%, 100%) respectively. Age groups of fishermen ranged between 20 to 70yrs; where age group 31- 40yr was dominant in Jebel Awlia and age group 41-50 was dominant in El-Mawrada. Six educational categories were recorded where primary education was dominant in the two location (48.8%, 44.4%) respectively; whereas, secondary education was the second in the two location (24.4%) and (27.8%) respectively. Part-time fishermen was dominant in both sites (34.1%, 11.1%); whereas full time fishermen was the lowest (4.9%, 16.7%). Most of fishermen were married (85%, 94%) in both sites; whereas unmarried fishermen was the lowest (14%, 5.6%). As regards to the fishermen's other activities results showed that most of them were not practicing any other activities (97.6%, 88.9%). Category of business owner was dominant. Dta showed that experience groups of fishermen in Jabel Awlia and El Mawrada fishery ranged between 5 to 40yrs. Where experience group 6 - 15yr was dominant in Jebel Awlia and group more than 35yr was dominant in El- Mawrada Experience group more than 35 yrs had the highest percentage in both sites; Most of fishermen in both sites did not get any training course (100%, 100%), except the fishermen of El- Mawrada who obtained some training in fish extinction (5.6%). Concerning fishers ownership, the results showed that owner list was dominant in both sites (85.4%, 66.7%); whereas, rent ship in both was the second (21.4%), (16.7%). For fish catchment by season results revealed that less than 5 ton was dominant in the tow locations (100%, 100%). Catch by season, income and fishing craft results in showed that category of fishermen who was able to catch less than 5 kg by season was dominant (100%) in Jabel Awlia and El Mawrada. In addition, most of fishermen income ranged between 1000 - 3000 pounds by year. However, the categories more than 2500 were dominant in both sites, followed by 1000 - 1500 pounds categories respectively. Result of skills and knowledge in fishery showed that fishermen had knowledge and skills in fishing gear and fishing draft respectively. Category had skills and knowledge in fishing time, fishing season and commercial species was dominant in both sites. On other side fish marketing skills and knowledge among fishermen in Jabel Awlia and El Mawrada was (51.2%, 77.8%) respectively. In addition, skills and knowledge of fishermen in fish processing presented as (51.2%, 61.1%) in both sites respectively.

**Keywords:** Fishery, Fishermen, Economic, Nile, Fishing.

## INTRODUCTION

The natural fisheries of Sudan are divided into two main sectors; the inland fisheries (fresh water fisheries) and the marine fisheries of the Red Sea. The inland fisheries are composed of the main Nile and its tributaries which are 6500 km long. And specially the reservoirs formed by the dams on the rivers; Jebel Aulia reservoir on the White Nile, Rosaries and Sennar reservoirs on Blue Nile, Khashm Algerba reservoir on Atbara River and Nuba Lake, which is the Sudan portion on Nasir reservoir. It lies in the northern part of Sudan, and it was formed by the construction of the Egyptian high dam south of Aswan. It is the richest source of fish in the Main Nile inside the Sudan, in addition to the Sud region at Upper White Nile (Awad Elkarim, 1999).

On the other hand, the marine fisheries are at the Sudanese coast line on the Red Sea, which extends to 720 km, and a continental shelf of about 98,000 km<sup>2</sup>, which is unsuitable for trawling due to its irregular coral beds (Sounness, 1978). This area is endowed with fine fishes, shelf fishes, 'crap' and crustacean 'shrimp and lobster'



(Osman, 1990), the total sustainable fish stock of Sudan is about 110,000 tons (Ministry of Agriculture Animal resources 1995).

Khartoum State covers an area of 21000 km<sup>2</sup> and the fish storage in it is estimated around 15,000 tons. But the amount exploited is not more than one thousand tons. The fish production is found in the fisheries inside Khartoum State in Jabal Awlia, Kalakla, Fetiah Al-Agaleen, El-Mawrada, the island of Al-Fitihab, Al-Sagai, Al-Sabalwaga and Al-Jeriah area on the Blue Nile (Abdal Mutalib, 2000). The process of handling and distribution of fish is carried by fishermen, traders and this confirmed with the case of Ethiopia (Degebassa, 2010).

The fish section in Khartoum State is characterized by being traditional in general and the ways and equipment of fishing did not find their chance to be modernized effectively. Add to that there are no enough means of storing, refrigeration and suitable transportation. On the other hand, the fish marketing activity is concentrated on only two markets out of the three fruit and vegetable central markets that exist in the state. Even in those two markets, there are very simple ways of preserving, showing and circulating the fish. In the state there are two stations for fish services which are regarded as a centre for the teams of the statistics. What is observed in these two centers is that there infrequency in the studies and researches that are concerned with the development of the fish section in the state (Ministry of Agriculture, 2004).

The main objective of this study is to investigate the economics and social characteristics of the fishermen in Jabal Awlia and Elmawrada at Khartoum state.

## MATERIAL AND METHODS

### Study area

Jabal Awlia: Jebel Awlia Dam reservoir (JADR) located at 32° 29'07.1" E and 15° 14'18.1" N, 40.6 Km<sup>2</sup> south of Khartoum; the dam elevation is 383m (Figure 1). It was constructed to control the flow of the Nile to aid the Aswan Dam in storing water for summer cultivation in Egypt.



FAO 2008



UNEP 2000

**Figure 1** - The location of Jebel Aulia Reservoir on the White Nile in Sudan [Source: adapted from FAO (2008) and UNEP (2000)].

**El-Mawrada:** El- Mawrada is considered as a rich region by fish stock and fish marketing in Sudan. This is located at White Nile at Omdurman at Khartoum State. It's one of the big fish market in Sudan and the coastal area is suitable locality for fishing, so that we found some parasite which affects a fish in this region.

### Data Collection

Raw data of this study were gathered through a questionnaire during summer season 2014. The questionnaire was designed to provide essential socio-economic information related to: sex, age, education levels, social status, time spending in fishing, other job beside fishing, fishermen activity, experience in fishery, training programs, owning fishing equipment, , ways of fishing used, catch by season ad income, fishing graft and skills and knowledge in fishery. 40 fishermen were questioned from Jabal Awlia and 18 fishermen were questioned from El-Mawrada.

## Statistical analysis

Descriptive analysis was done for analyzing the raw data of the study by using Excel Microsoft Software 2003.

## RESULTS AND DISCUSSION

This study was conducted to investigate socioeconomic characteristics of fishermen in Jabel Awlia and El-Mawrada areas at Khartoum State.

Social data Table 1 showed that age groups of fishermen in Jabel Awlia and El Mawrada fishery ranged between 20 to 70 yrs. Age group less than 20 yrs had the lowest percentage as 7.3% in Jabel Awlia and 5.6% in El Mawrada; whereas, age group 31- 40yrs was the dominant (22%) in Jabel Awlia and the age group 51-60 and above 60yrs was dominant in El Mawrada (27%), followed by age group 41-51 and 51-60yr (19.5%) in Jabel Awlia and age group 31-40yr in El Mawrada (22.2%). These results reflect that most fishermen who practiced fishing activity were youth and this dominance may also be due to flood season, during which rate of fishes are high that may attract youth to get benefit and probably they are able to practice fishing during strong wind and high waves of the water. This result is in agreement with results of Hamza (1981). In contrary, age group 60-70yrs may represent the rate of the professional fishermen who practice fishing during whole year. This result is in harmony with results of FDKS (2003).

**Table 1 - Distribution of fishermen according to their personal characteristics**

Variable	Jabel Awlia		Elmawrada	
	N	%	N	%
<b>sex</b>				
Male	40	97.6	18	100
Female	1	2.4	0	0
<b>age</b>				
Less than20	3	7.3	1	5.6
21-30	7	17.1	0	
31-40	9	22	4	22.2
41-50	8	19.5	3	16.7
51-60	8	19.5	5	27.8
More than60	6	14.6	5	27.8
<b>Educational level</b>				
Illiterate	8	19.5	4	22.2
Khalwa	0	0	1	5.6
Primary	20	48.8	8	44.4
Secondary	10	24.4	5	27.8
University	3	7.3	0	0
Above university	0	0	0	0
<b>social status</b>				
Married	35	85	17	94.4
Unmarried	6	14	1	5.6
<b>Full or part time</b>				
Full time	2	4.9	3	16.7
Part time	14	34.1	2	11.1

General educational level (48.4%) in Jabel Awlia and (44.4%) in El Mawrada, high educational level (7.3%), (0%) in Jabel Awlia and El Mawrada respectively and illiteracy (19.5%) in Jabel Awlia and (22.2%) in El Mawrada; the general educational was dominant followed by secondary and illiteracy this result is under line with result of Mohammed (2004) who reported that most fishermen of the state have basic education of 47%; whereas, the illiterate fishermen constituted 31.7%.

Economic results showed that two categories of fishermen (professional fishermen, part-time fishermen) were presented with different percentages such as: 4.9%, 16.7% and 34.1%, 11.1% respectively. Category of part – a time fisherman was dominant and explains why age group 31- 40yr was one of the dominance age group of fishermen In addition, most of fishermen was married (85%),(94.4%); in Jabel Awlia and El Mawrada respectively. This result also harmonizes with results of Mohammed, M. O. (2006).

Other activity data in Table 2 showed that social practice of fishermen in Jabel Awlia and El Mawrada fishery is different. Not practice group had the highest percentage as (97.6%) in Jabel Awlia and (88.9%) in El Mawrada; followed by member of fishermen society (2.4%) in Jabel Awlia and in El mawrada (11.1%). Also other activity

results showed that four categories of other job of fishermen (farmer, officer, worker, business owner) were presented with different percentages such as: 9.5%, 0% and 7.3.1%, 16.7% and 4.9%, 5.6% and 7.3%, 22.2% and 0%, 0% respectively. Category of business owner was dominant followed by officer and other job group had lowest percentage (0%). This result also harmonizes with results of Mohammed, 2006); FDKS (2004).

**Table 2 - Distribution of fishermen according to their other activity**

Variable	Jabel Awlia		ELmawrada	
	N	Percentage	N	Percentage
<b>Social activity</b>				
Not practice	40	97.6%	16	88.9
Member of Fishermen society	1	2.4%	2	11.1
Member of a cooperative society	0	0	0	0
Member of the popular committee	0	0	0	0
Member of board parents	0	0	0	0
Other	0	0	0	0
<b>Other job</b>				
Farmer	4	9.8	0	0
Officer	3	7.3	3	16.7
Worker	2	4.9	1	5.6
Business owner	3	7.3	4	22.2
Other	0	0	0	0

Data in Table 3 showed that experience, training course and owner in fishery; experience groups of fishermen in Jabel Awlia and Elmawrada fishery ranged between 5 to 40yr. Experience group more than 35 yr had the highest percentage as 26.8%in Jabel Awlia and 38.9% in Elmawrada; followed by experience group 6-15 yr (29.3 %) in Jabel Awlia and in Elmawrada (27.8%).

Also training course results showed that six categories of training course of fishermen (fishing gear and graft, fishing time, fishing season, fish marketing, fish extinction and fish processing) were presented with the same percentages all as: (0%) except fish extinction (5.6%) in Elmawrada. In case of their owner the result showed that owner list group was dominant (85.4%), and (66.7%) in Jabel Awlia and Elmawrada respectively, followed by rent group (21.4%) in Gabel Awlia and (16.7%) in Elmawrada, but partnership group had lowest percentage (0%) in the tow location. This result is similar to result of (Mohammed, M. O. 2004) who reported that 90% of whole fishermen have owned their fishing gear and have no formal training to do fishing.

**Table 3 - Distribution of fishermen according to their experience, training programmers and owner in fishers.**

Variable	Jabel Awlia		ELmawrada	
	N	%	N	%
<b>Experience</b>				
Less than 5 year	10	24.4	1	5.65
6 - 15	12	29.3	5	27.8
16 - 25	5	12.2	2	11.1
26 - 35	3	7.3	3	16.7
More than 35	11	26.8	7	38.9
<b>training programmers</b>				
Fishing gear &graft	0	0	0	0
Fishing time	0	0	0	0
Fishing season	0	0	0	0
Fish marketing	0	0	0	0
Fish extinction	0	0	1	5.6
Fish processing	0	0	0	0
Other	0	0	0	0
<b>Their owner</b>				
Owner list	35	85.4	12	66.7
Free hold	2	4.9	1	5.6
Rent	3	21.4	3	16.7
Partner ship	0	0	0	0
Owner	0	0	1	5.6
Other	1	2.4	1	5.6

Catch by season, income and fishing craft results in table (4); showed that four categories of fishermen according to their catch by season (less than 5 kg, 5 - 7 kg, 7 - 9 kg and more than 9 kg) .Category of less than 5 kg by season was dominant (100%) in Jabel Awlia and Elmawrada but the other categories had (0%) in the tow location respectively and that explains why fishermen had low income by season. In addition, most of fishermen income ranged between 1000 – 3000 pounds by year. The categories more than 2500 was dominant (41.5%) in Gabel Awlia and (50%) in Elmawrada, followed by 1000 – 1500 pounds categories (29.3%), (38.9%) in Gabel Awlia and Elmawrada respectively. Categories 1501 – 2000 had lowest percentage (4.9%) in Gabel Awlia and (0%) in Elmawrada. This result is semi to result of Abd El-Rahaman, (2003) who recorded that the total annual yield of JADR was estimated at 115.732kg and the maximum sustainable yield was 90.706kg as in. Also fishing of craft using in fishing results showed that four type of graft use by fishermen (boat, vessel, lynch and other) were presented with different percentages such as: 56.1%, 50% and 14.6%, 5% and 2.4%, 0% and 26.8%, 0% respectively. Category of boat was dominant (56.1%), (50%) followed by other group (26.8%), (0%) in two location followed by vessel group (14.6%), (5%) respectively and the lynch group had lowest percentage (2.4%), (0%) in Jabel Awlia and Elmawrada.

**Table 4 - Distribution of fishermen according to their catch by season, income and fishing craft.**

Variable	Jabel Awlia		ELmawrada	
	N	%	N	%
<b>Catch by season</b>				
Less than 5 ton	41	100	18	100%
5 - 7 ton	0	0	0	0
7 - 9 ton	0	0	0	0
More than 9 ton	0	0	0	0
<b>Income (pound)</b>				
Less than 1000	8	19.5%	1	5.6
1000 - 1500	12	29.3%	7	38.9
1501 - 2000	2	4.9%	0	0
2001 - 2500	2	4.9%	1	5.6
More than 2500	17	41.5%	9	50
<b>Fishing craft</b>				
Boat	23	56.1	9	50
Vessel	6	14.6	9	50
Lynch	1	2.4	0	0
Other	11	26.8	0	0

Result of skills and knowledge in fishery in Table 5 showed that fishermen had skills and skills in fishing gear people say yes ranged (78.4), (100%), fishing draft fishermen say yes 70.7%, 94.4% respectively. Category had skills and knowledge in fishing time, fishing season and commercial species was dominant people say yes approach (100%) in Jabel Awlia and Elmawrada and that explains why fishermen had a good experience in fishery. On other side fish marketing skills and knowledge in Jabel Awlia percentage (51.2%) from the fishermen say yes and 34.1% say No, but in Elmawrada (77.8%) say Yes and 0% say No. In addition, skills and knowledge of fishermen in fish processing presented as (51.2%), (61.1%) of the fishermen say Yes and (34.1), (0%) say No in Jabel Awlia and Elmawrada respectively. Skills and knowledge in fishery or other fields accrued by learning, practice and dependent on years of experience.

**Table 5 - Distribution of fishermen according to their skills and knowledge in fishery**

Variable	Jabel Awlia				Elmawrada			
	Yes	Percentage	NO	Percentage	Yes	%	NO	%
Fishing gear	32	78.4	0	0	18	100	0	0
Fishing graft	29	70.7	0	0	17	94.4	0	0
Fishing time	41	100	0	0	18	100	0	0
Fishing season	41	100	0	0	18	100	0	0
Fish marketing	21	51.2	14	34.1	14	77.8	0	0
Fishing area	35	85.4	2	4.9	16	88.9	4	22.2
Fish processing	21	51.2	14	34.1	11	61.1	0	0
Commercial species	41	100	0	0	18	100	0	0



Finally Both inland and marine fisheries resources play an important role in food security and export trade especially in developing countries. In Sudan fisheries, especially those in Khartoum state, the profession of fishing has traditionally been learned by mimicking where a few of them have learned by concerned institute (Mohammed, 2006).

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# THE PERFORMANCE OF GROWING PIGS FED DIETS CONTAINING DIFFERENT LEVELS OF SODIUM HYDROXIDE-TREATED SHEANUT KERNEL CAKE

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**ABSTRACT:** The aim was to investigate the use of sodium hydroxide treated (0.01M) Sheanut kernel cake (TSNKC) as a feed ingredient in growing pig diets. The growth rate, feed intake and feed to gain ratio of growing pigs fed diets containing 100 (100USNKC/control), 100 (100TSNKC), 150 (150TSNKC) or 200 (200TSNKC) g kg<sup>-1</sup> of sodium hydroxide treated Sheanut kernel cake was investigated in two feeding trials set up as completely randomized designs with 4 treatments replicated 3 times and lasting 120 days. The two feeding trials were combined and analyzed as a split-plot, with the trials being the main plot and the experiment within the trial as the sub plot. Treatment 100TSNKC was significantly different ( $P < 0.05$ ) from treatment 200TSNKC. Treatments 100USNKC and 150TSNKC were not significantly different ( $P > 0.05$ ) from either 200TSNKC or 100TSNKC. Total feed intake for 200TSNKC was significantly lower ( $P < 0.05$ ) as compared to the other treatments but feed to gain ratio was not significantly different ( $P > 0.05$ ). It was concluded that it is possible to include up to 150 g kg<sup>-1</sup> sodium hydroxide treated Sheanut kernel cake in growing pig diets. Future work will need to look at ways of improving the palatability of sodium hydroxide treated Sheanut kernel cake and also look at carcass analysis and blood profiles.

**Keywords:** Sheanut, Sodium Hydroxide Treatment, Sheanut Kernel Cake, Growing Pigs, Feeding Trial.

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## INTRODUCTION

The Shea tree (*Vitellaria paradoxa* Gaertner) is a parkland woody tree commonly not cultivated. It grows wild extensively in the dry savannah belt of West Africa, stretching from Senegal in the west to Sudan in the east (Bernice, 2011). In Ghana, the Guinea Savanna, Forest Savanna and Sudan Savanna, which cover about two-thirds of the country's land Mass, is home to the Shea tree (Abiw, 1990).

The tree plays an important role in sustaining the livelihoods of people living in rural areas. The fruit of the Shea tree consists of a green fleshy mesocarp, which has a high nutritional value and contains between 0.7 to 1.3 grams of protein and 41.2 gram of carbohydrate (Bernice, 2011). Within the mesocarp is a kernel which is a rich source of fat and the most important product of the Shea tree is Shea butter which is extracted from the dried kernels (IPGRI, 2006). The butter is used in cooking (Aguzue et al., 2013), as a body cream, fuel in rural lamps and in medicinal preparations (Yeboah, 2009). Presently, Shea is exported from Africa to France, Great Britain, the Netherlands, Denmark, North America and Japan (Elias and Carney, 2007), where it is used in cosmetics and as a cocoa butter substitute in confectionary.

The kernel contains up to about 60% of fat (Shea butter) and the residue after the extraction of butter from the kernel has been described as Sheanut kernel cake, and is a major by-product of the Sheanut industry. It is rich in carbohydrates and protein, but the presence of anti-nutritional factors, mainly theobromine and tannins, limits its use as an animal feed ingredient (Gohl, 1981; Okai and Bonsi, 1989; Okai, 1990; Rhule, 1995, 1999; Annongu et al., 1996 and Atuahene et al., 1998, Dei et al., 2008). Oddoye et al. (2012) investigated the use of physical treatments as a way of reducing the level of some of these anti-nutritional factors, and reported that treatment with sodium hydroxide (0.01M) gave the most promising results. As a follow up to that experiment, it was decided to feed sodium hydroxide treated (0.01M) Sheanut kernel cake to growing pigs, to assess whether the treated material could be fed at a higher level than reported in literature.

## MATERIAL AND METHODS

### Location

The experiment was carried out at the Worakese Plantation of the Cocoa Research Institute of Ghana. Sheanut kernel cake was procured from Ghana Nuts Company limited in Techiman, in the Brong Ahafo Region of Ghana.

### Treatment with Sodium hydroxide

The cake was spread in a thin layer on a concrete drying platform. A solution of sodium hydroxide (0.01M) was applied to the cake using a knapsack sprayer, until the material was thoroughly wet with the solution. A knapsack sprayer (17 liters) treated about 5 bags of Shea kernel cake weighing 50 kg each. The treated material was allowed to sun-dry for two days. At the end of each day the material was collected to prevent being wet by overnight rain and was spread out again to dry the following day.

### Feed ingredients

Other feed ingredients like fish meal, tuna meal, etc. were procured for accredited local dealers.

### Feeding trials

Two trials were run for one hundred and twenty days each; the first from February 15th until June 24th, 2013 and the second from 15th August until 22nd December, 2013. Both trials included an initial ten-day adjustment period. Each trial was laid out as a completely randomized design with 4 treatments and 3 replicates making a total of 12 experimental units. Twelve Large White, growing pigs were used for each trial. Pigs were individually housed in twelve pens. Pens were roofed (aluminum) and were constructed with cement blocks and had concrete floors with a rough finish.

A Priori, it was decided that rather than have a conventional control for comparison (standard diet fed to growing pigs), a treatment with untreated Shea kernel cake would serve as the control. Other treatments would then incorporate sodium hydroxide treated Sheanut kernel cake at increasing levels. For each trial, pigs were balanced for age, sex and litter and randomly allocated to one of four (4) treatments having 100 g Kg<sup>-1</sup> untreated Sheanut kernel cake (100USNKC/control), 100 g Kg<sup>-1</sup> sodium hydroxide treated Sheanut kernel cake (100TSNKC), 150 g Kg<sup>-1</sup> sodium hydroxide treated Sheanut kernel cake (150TSNKC) and 200 g Kg<sup>-1</sup> sodium hydroxide treated Sheanut kernel cake (200TSNKC). Diets were formulated to be iso-energetic (metabolisable energy value for Sheanut cake of 12.6 MJ Kg<sup>-1</sup> as estimated by Dei et al., 2007 was used in calculations) and iso-nitrogenous and were based on standard growing pig diets. All feeds contained unconventional feed ingredients like cocoa pod husk and reject cashew kernel meal. As the level of TSNKC increased, the level of other feed ingredients had to be adjusted to ensure that diets were approximately iso-energetic and iso-nitrogenous. After some juggling it became apparent that the simplest and most cost effective way of doing this was to reduce the level of wheat bran in the diet.

Pigs were fed an allowance, equivalent to 5% of their body weight, once a day and water was provided ad libitum. Any feed left over at the beginning of the next day was weighed and subtracted from that which had been fed the previous day to determine feed intake. Feed allowance was adjusted at the end of each month after the pigs had been weighed. Samples of each feed were subjected to proximate chemical analysis (AOAC, 2000). The method of Akaninwor and Okechukwu (2004) was used to determine tannin concentration in the feeds.

Feed intake was recorded daily for each pen and pooled for a month (28 days). This was then used in the computation of average daily feed intake. Similarly, weights taken at the end of every month were used in the computation of average daily weight gain. The average daily feed intake, divided by the average daily weight gain was calculated as the feed to gain ratio, that is, the weight of feed needed to produce one kilogram of gain.

The effects of the various treatments on average daily weight gain, average daily feed intake and feed to gain ratio were investigated using analysis of variance (GENSTAT), with the initial weight of pigs serving as a covariate. A Priori, and on the advice of the Institution's Biometrician, it was decided to run two trials as the pens could only hold 12 animals at a time, which would make for a low error degrees of freedom. The results of the two trials were, therefore, combined and analyzed as a split-plot experiment with the trials being the main plots and the data collected within each trial being the sub plots.

## RESULTS

The composition of experimental feeds and the results of proximate analysis are shown in Tables 1 and 2, respectively. Means for the various treatments for average daily weight gain, average daily feed intake, and feed to

gain ratio are shown in Table 3. Analysis of variance revealed a significant difference ( $P < 0.05$ ) between treatments with respect to average daily gain. The treatment 100TSNKC was significantly different ( $P < 0.05$ ) from treatment 200TSNKC. Treatments 100USNKC and 150TSNKC were not significantly different ( $P > 0.05$ ) from either 200TSNKC or 100TSNKC. Total feed intake for 200TSNKC was significantly lower ( $P < 0.05$ ) as compared to the other treatments. There was also a significant ( $P < 0.05$ ) interaction in that intake of 200TSNKC was much lower during the first trial. The depression in feed intake did not significantly affect the feed to gain ratio as means were not significantly different ( $P > 0.05$ ) from each other.

**Table 1 - Composition of growing-finishing pig feeds**

Ingredients (g kg <sup>-1</sup> )	Experimental feeds			
	100 USNKC (ctrl)	100 TSNKC	150 TSNKC	200 TSNKC
Untreated Shea cake	100	–	–	–
Treated Shea cake	–	100	150	200
Wheat bran	245.75	245.75	195.75	145.75
Reject cashew kernels	300	300	300	300
Cocoa pod husk	250	250	250	250
Soya bean meal (local)	50	50	50	50
Tuna meal	30	30	30	30
Oyster shell	10	10	10	10
Dicalcium phosphate	5	5	5	5
Common salt	5	5	5	5
Vit./min. premix	1.25	1.25	1.25	1.25
Synthetic lysine	1	1	1	1
Synthetic methionine	1	1	1	1
<sup>a</sup> Mycofix	1	1	1	1
Total	1000	1000	1000	1000
<b>Calculated Analysis</b>				
Metabolizable Energy (MJ Kg <sup>-1</sup> )	12.4	12.4	12.6	12.7
Crude protein (g kg <sup>-1</sup> )	182.3	182.3	182.7	183.1
Lysine (g kg <sup>-1</sup> )	13.0	13.0	12.7	12.4
Methione + cystine (g kg <sup>-1</sup> )	54.2	54.2	5.2	4.9
Calcium (g kg <sup>-1</sup> )	8.6	8.6	8.5	8.4
Available phosphorus (g kg <sup>-1</sup> )	4.9	4.9	4.3	3.7
Cost (GH¢ metric tonne <sup>-1</sup> )	580.00	630.00	610.00	590.00

Notes: 1GH¢ = 0.53USD as at December 31, 2012. <sup>a</sup>Mycofix Select 3.0 (BIOMIN GmbH, Herzogenburg, Austria) is a commercial mould fixing agent which is added to feeds at a rate of 1 Kg per 1000 Kg of feed. It binds mycotoxins *in vivo* preventing them from causing harm to the animals. 100 USNKC - 100 g kg<sup>-1</sup> untreated Sheanut kernel cake in feed (control), 100 TSNKC - 100 g kg<sup>-1</sup> treated Sheanut kernel cake in feed, 150 TSNKC - 150 g kg<sup>-1</sup> treated Sheanut kernel cake in feed, 200 TSNKC - 200 g kg<sup>-1</sup> treated Sheanut kernel cake in feed.

**Table 2 - Proximate analysis and tannin content of experimental feeds and untreated and sodium hydroxide treated Sheanut kernel cake**

Parameter	Experimental feeds					
	100USNKC	100TSNKC	150TSNKC	200TSNKC	USNKC <sup>+</sup>	TSNKC <sup>+</sup>
Dry matter (g kg <sup>-1</sup> )	988	988	992	989	925	970
Organic matter	938	932	956	948	945	915
Crude protein	175	169	182	166	178	175
Ether extract	38	40	43	45	25	50
Crude fibre	70	70	74	77	114	124
Tannin (mg kg <sup>-1</sup> )	3.16	2.12	2.53	2.92	20.0	6.0

<sup>+</sup> Data from Oddoye et al. (2012).

**Table 3a - Average daily weight gain (g day<sup>-1</sup>) for pigs on experimental diets**

Trial	Treatments				
	100USNKC	100TSNKC	150TSNKC	200TSNKC	Means
1	0.42	0.40	0.39	0.33	0.38
2	0.36	0.45	0.40	0.34	0.39
Means	0.39	0.43	0.39	0.33	–

SED: Trial 0.014, Treatment 0.028, Trial\*Treatment 0.037; when comparing means with the same level(s) of Trial; 0.040



**Table 3b - Total feed intake (kg) for pigs on experimental diets**

Trial	Treatments				Means
	100USNKC	100TSNKC	150TSNKC	200TSNKC	
1	218.3	221.2	210.2	160.4	202.5
2	216.3	219.7	212.0	199.0	212.0
Means	217.3	220.4	211.6	179.7	–

SED: Trial 3.910, Treatment 5.780, Trial\*Treatment 8.080; when comparing means with the same level(s) of Trial; 8.170

**Table 3c - Feed conversion ratio for pigs on experimental diets**

Trial	Treatments				Means
	100USNKC	100TSNKC	150TSNKC	200TSNKC	
1	3.97	4.16	4.16	3.67	3.99
2	4.58	3.71	4.07	4.59	4.24
Means	4.27	3.94	4.12	4.13	–

SED: Trial 0.125, Treatment 0.329, Trial\*Treatment 0.422; when comparing means with the same level(s) of Trial; 0.465

## DISCUSSION

There was an improvement in the performance of pigs when sodium hydroxide treated Sheanut kernel cake was used in the diets of growing pigs. Levels of up to 150 g kg<sup>-1</sup> in the feed (150TSNKC), an increase of 50% over the control, gave comparable growth rates with the control. Rhule (1999) reported similar improvements in performance when he fed detoxified Sheanut cake to pigs. The big drop in feed intake of pigs on 200TSNKC in trial 1 remains a mystery and may be due to inaccurate recording of experimental data or may have been caused by improper treatment of the test material.

The general trend seemed to be one of decreasing feed intake with increasing level of Sheanut kernel cake, even though it had been treated with sodium hydroxide in a bid to reduce the content of anti-nutritional factors. It had been expected that the sodium hydroxide treatment would lead to an increased intake but this did not prove to be the case. In a similar experiment with broilers, in which detoxification was achieved by fermentation with *Aspergillus niger* or *Ceriporiopsis subvermispota*, Dei et al. (2008), however, reported that feed intakes, weight gain and feed conversion efficiencies improved when birds were fed with the detoxified material as compared to the unfermented Shea nut meal. It has been suggested that sodium hydroxide treatment itself may cause palatability problems as evidenced in feeding of sodium hydroxide treated material to white albino rats in the laboratory (Sackeyfio, JM, Personal communication, 2015). The levels used in the previous experiment (Oddoye et al., 2012) were selected from literature and there is the need to go back to the drawing board to determine whether a lower concentration of sodium hydroxide can be used to achieve an appreciable reduction in the level of anti-nutritional factors. There would also be the need to do a complete analysis of all anti-nutritional factors in Sheanut kernel cake and investigate the effects of sodium hydroxide treatment on them. It has been suggested that the saponins in Shea kernel cake may have reacted with sodium hydroxide to form soaps, which will make the material unpalatable (Agyente-Badu, K, Personal communication. 2015). There may also be the need to batch test all sodium hydroxide treated Sheanut kernel cake to make sure that they have been well treated. Sampling of blood to ensure that feeding of sodium hydroxide treated Sheanut kernel cake will not give any health problems and the analysis of carcasses produced when the material is fed will also need to be carried out. The level of tannins assayed in the complete feeds, as compared to what would be expected if tannins were coming from Shea kernel cake alone, was quite high and is indicative of the fact that some of the other feed ingredients may be adding to the tannin load in the feed. There is a paucity of literature on the feeding of Sheanut kernel cake to pigs and other livestock. This is probably because of the high level of anti-nutritional factors making most researchers to decide that it is a material that does not have a future as far as livestock feeding is concerned. As such, we were not able to compare our work adequately with what others have done.

In conclusion, sodium hydroxide as a method of treatment of Sheanut cake to reduce anti-nutritional factors holds promise. Palatability is, however, a problem and this will have to be rectified if more than 150 g kg<sup>-1</sup> is to be included in growing pig feeds.

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# THE USE OF CASSAVA ROOT MEAL AS A PARTIAL REPLACEMENT FOR CORN IN DIETS FOR ALBINO RATS

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**ABSTRACT:** The experiment was carried out to determine if partial replacement of maize with cassava root meal (CRM) in diets for albino rats will have an effect on performance, organ characteristics and blood parameters. Twenty five Sprague Dawley albino rats (F344 strain), initial body weight ( $216 \pm 8$ g) were randomly assigned to five treatments (T1 to T5) in a completely randomized (CRD) arrangement. T1 was the control and contained zero CRM. T2 and T3 contained 30% CRM, while T4 and T5 contained 45% CRM. These levels of inclusion represented 50 and 75% replacement of corn in the diets respectively. T2 and T4 had 0.15% methionine (Met) while T3 and T5 had 0.3% Met. The rats were each fed a single diet for 28d. Average daily feed intake (ADFI) of rats on T1 was lower ( $P < 0.05$ ) than that for rats on T4 and T5 (12.12g vs. 12.77 and 12.64g) respectively. For diets with the same level of CRM, those with 0.3% Met had a lower consumption than those with 0.15% Met. There were no differences ( $P > 0.05$ ) in average daily gain (ADG) and feed conversion efficiency (FCE). Similarly there were no differences ( $P > 0.05$ ) in carcass, viscera and other internal organ weights. Results of this trial indicate that albino rats can tolerate diets with added CRM (45% of the diet) with no adverse effects on growth and internal organ characteristics. Future work will need to look at the possibility of using CRM at similar or higher levels in diets for growing pigs.

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## INTRODUCTION

Feed represents about 65-70% of the total cost of intensive animal production (Tewe, 1997). In monogastric feeds, energy providing ingredients make up approximately 60% of the ration (Oppong-Apene, 2013). In developing countries most of the major energy providing ingredients used in monogastric diets like maize and wheat are imported at great cost and with hard-earned foreign exchange. With rising populations and increasing income levels, meeting the accompanying demand for protein-rich diets at an affordable cost becomes a challenge for farmers.

Monogastrics (broilers and pigs) have the ability to grow fast and also turn plant material into animal protein. However due to the high cost of imported energy-providing feed ingredients, it is imperative that monogastric farmers look beyond the traditional cereal grains, and depend more on alternatives (Chauynarong et al., 2009). One such alternative is cassava root meal (CRM) which grows abundantly under tropical conditions.

The use of CRM in diets for monogastrics has provided conflicting results in terms of animal performance and productivity (Adeniji and Balogun, 2003; Obikaonu and Udedibie, 2006). Although CRM is high in starch content (60-70%) the protein content is very low ranging from approximately 2.5 to 3.25% (Garcia and Dale, 1999; Nortey et al., 2013). It is recommended to include a good source of protein such as soybean meal or fishmeal and synthetic amino acids (particularly methionine), in diets based on CRM since it is particularly deficient in this amino acid (Rakangtong and Bunchasak, 2010). In addition to a low protein (and amino acid) content, CRM is high in non-starch polysaccharides (NSP) and other toxins like cyanogenic glucosides, tannins, and phytates (Teguia and Beynen, 2004). Despite these challenges, CRM is a promising feed ingredient that can be used in monogastric feeds. When well formulated, CRM can replace maize in diets for poultry (Chauynarong et al., 2009; Nortey et al., 2013) and pigs (Lan et al., 2008). Increased use of CRM in monogastric diets will ultimately result in a lower cost of animal protein production to serve the needs of a rapidly growing middle class population in the developing world.

The hypothesis of this study therefore was that cassava root meal can partly replace maize in diets for Sprague-Dawley outbred albino rats without negatively affecting growth, carcass composition and blood metabolite profiles. The objectives were to investigate the efficacy, growth related responses, carcass characteristics and blood profiles of albino rats fed two dietary levels of cassava root meal. Similar to other trials (Okai et al., 2013; Ogunleye and Omotoso, 2005) the rat is being used as a model for the pig.

## **MATERIAL AND METHODS**

The experiment was carried out at the Noguchi Memorial Institute of Medical Research (NMIMR). The institute was established in 1979 in a building funded by the Japanese government to serve as a monument in memory of Dr Hideyo Noguchi, a Japanese medical scientist who died in Accra in May 1928 while investigating yellow fever. The institute provides a base for medical co-operation programmes between Ghanaian and Japanese scientists and a centre for conducting medical research relevant to Ghana's needs.

### **Processing of whole cassava root meal**

Fresh cassava roots with a moisture content of about 80% were obtained from the open market, chopped into small bits with the skins still on, and sun-dried for about seven days to approximate moisture content of 11.5%. The sun-dried product was then ground through a hammer mill to a particle size of approximately 500 microns, stored in a cool, dry and airy environment prior to incorporation into feed.

### **Experimental diets**

Five experimental diets were formulated with varying levels of CHP as follows: T1 was the control diet with zero CRM and with added methionine (Met) at a rate of 0.15%; T2 was similar to T1 but had 50% of the total maize content replaced with CRM; T3 was the same as T2 but with twice the level of added methionine (0.3%); T4 had 75% of the total maize content of T1 replaced with CRM and with 0.15% total Met; T5 was the same as T4 but with 0.3% added Met. All the diets were pelleted before being fed to the experimental animals.

### **Management of experimental animals**

The animal protocol used followed principles recommended by the Institutional Animal Care and Use Committee of the Noguchi Memorial Institute for Medical Research, University of Ghana. Thirty albino rats (Sprague-Dawley F344 strain) supplied by the Animal Experimentation Unit of NMIMR were placed in their individual cages and allowed to acclimatize to their new environment for two weeks. On day 15, twenty five rats (Initial body weight:  $198 \pm 5$ g) were selected to be used as test animals. These were randomly assigned in a Completely Randomized Design (CRD) with five replicates per treatment to one of five diets and allowed a further seven days to get used to the feed before the start of experiment. The rats were housed in individual wire-mesh cages with plastic coated floors, each measuring 20cm x 24cm x 20cm (Length x Breadth x Height) and had freedom of movement. The cages were placed randomly on aluminum shelves. Each cage had a metallic feeding trough and nipple drinker provided. Rats were individually weighed at the start of the trial, and subsequently on a weekly basis. Rats in each replicate were given a known amount of feed and water daily. Feed left over after 24 hours was weighed to determine average daily feed intake (ADFI). The whole feeding and growth trial lasted for 28 days.

### **Carcass and haematological analysis**

On day 29, blood from each anaesthetized rat was collected separately by holding the rats vertically and placing a 10 ml needle into either the tail artery or vein. The blood (about 3ml) was collected into a 4mL sample tube (Surgifield Medicals, Meddlessex, England) using  $K_3$  EDTA as the anticoagulant. For serum profile, about 3 ml of blood was collected into 5mL Serum-separator vacuum tubes (Surgifield Medicals, Meddlessex, England). Both samples were analysed the same day for haematological and coagulation parameters. Next all the anesthetized rats were killed by cervical dislocation and internal organs (i.e.) viscera, liver, kidney lungs, heart and spleen were removed and weighed. The anaesthesia used was chloroform. Haematology parameters were analysed using an Advia 120 (Siemens AG, Munich, Germany) analyser. Parameters evaluated were: white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PLT)

### **Chemical analysis**

Proximate analysis was carried out on both the ingredients and experimental diets according to methods outlined by the Association of Official Analytical Chemists, (AOAC, 1995). For Ca and P determinations, the methods according to James (1996) and AOAC (1995) were used.



### Statistical analysis

All the data gathered were subjected to statistical analysis using the Generalized Linear Model procedure of the Statistical Analysis Systems Institute (SAS, 1999). Significant differences among means were separated using the Student Newman-Kuels (SNK) Test.

## RESULTS

Table 1 shows the analyzed chemical composition of whole CRM used in this trial. Table 2 shows the composition and calculated nutrient values respectively of the experimental diets.

### Growth traits

Average daily feed intake (ADFI) of rats on T4 (45% CRM + 0.15% Met) was significantly more than ( $P < 0.05$ ) the ADFI of rats fed the control diet (T1), and those on T3 (30% CRM + 0.3% Met). Also rats on T5 (45% CRM + 0.3% Met) ate significantly more feed ( $P < 0.05$ ) than rats on the control diet (Table 3). There were no significant differences ( $P > 0.05$ ) in ADG and FCE among all the treatments.

### Organ weights and haematological parameters

There were no significant differences ( $P > 0.05$ ) in organ weights (Table 3) and haematological parameters among all the treatments (Table 4).

**Table 1 - Analysed chemical composition of cassava root meal used in trial (as is basis)**

Parameter	Value (%)
Dry matter	88.12
Crude protein	3.32
Acid detergent fiber	2.14
Neutral detergent fiber	5.85
Ether extract	0.71
Ash	2.22
Calcium	0.16
Phosphorous	0.12

**Table 2 - Composition and calculated analysis of the experimental diets**

Ingredients	Control	30% CRM <sup>1</sup> 0.15% Met <sup>2</sup>	30% CRM 0.3% Met	45% CRM 0.15% Met	45% CRM 0.3% Met
Corn	60	30	30	15	15
Cassava root meal	0	30	30	45	45
Soya bean meal	22.5	22.5	22.5	22.5	22.5
Wheat bran	15.7	15.7	15.55	15.7	15.55
Oyster shells	1	1	1	1	1
Common salt	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.30	0.15	0.30
Total	100	100	100	100	100
<b>Calculated Analysis</b>					
ME (MJ/Kg)	11.75	11.57	11.59	11.48	11.50
CP %	19.96	16.99	17.10	15.50	15.61
CF %	3.43	4.15	4.14	4.51	4.5
Total Lysine %	1.09	1.00	1.00	0.96	0.96
Total Methionine %	0.42	0.36	0.48	0.33	0.45
Ca %	0.56	1.25	1.25	1.59	1.59
P %	0.62	0.80	0.8	0.89	0.89

\*SEM; <sup>1</sup>Cassava Root Meal; <sup>2</sup>Methionine;

## DISCUSSION

### Growth traits

Generally monogastrics will eat more of a low-nutrient dense diet and vice versa, gut fill permitting. This phenomena has been observed in both birds (Plavnik et al., 1997; Leeson, 2000; Veldkamp et al., 2005; Nahashon

et al., 2006) and pigs (Lan et al., 2007; Madrid et al., 2013). This is because monogastrics will eat to meet their requirements for particular nutrients. Once the requirement is met, intake is reduced. In this trial, the dietary nutrient densities were progressively (but gradually) lowered as the level of CRM increased in the diet. Thus the fact that rats on T1 ate the least amount of feed is in line with previous findings. Between diets with either 30% or 45% CRM, those with 0.3% Met were more balanced than those with 0.15% Met, hence the observed numerical decreases in ADFI when the Met levels were doubled at the different CPH inclusion levels. Also the fact that rats on T4 ate the most feed may be a direct reflection of the fact that this diet is the most unbalanced in terms of the essential amino acid, methionine.

### Organ weights and haematological parameters

The observed similarities in organ weights between the control and the other dietary treatments is an indication that the presence of CRM in the diet presented no adverse effects on the internal organs of the rats. With increasing levels of CRM, the fibre contents tended to increase. Generally animals fed on diets that are high in fibre, tend to have more developed empty gastro-intestinal tracts (GIT) (Jørgensen et al., 2006). This is due to heavier musculature of the intestinal walls which is a direct response to the greater amount of feed that the animal will necessarily have to hold in the GIT.

**Table 3 - Effect of cassava root meal on growth parameters and internal organ weight**

Parameter	Control	30% CRM <sup>1</sup> 0.15% Met <sup>2</sup>	30% CRM 0.3% Met	45% CRM 0.15% Met	45% CRM 0.3% Met	SEM*	P-Value
ADFI (g)	12.12 <sup>c</sup>	12.47 <sup>abc</sup>	12.30 <sup>bc</sup>	12.77 <sup>a</sup>	12.64 <sup>ab</sup>	0.16	0.03
ADG (g)	3.70	3.57	3.63	2.93	3.47	0.41	0.70
FCE	0.31	0.29	0.29	0.23	0.27	0.03	0.47
<b>Organ Weights</b>							
Carcass	250	258	250.8	273.5	260	9.07	0.39
Viscera	50.55	56.16	51.84	53.8	59.34	3.15	0.32
Kidney	1.43	1.46	1.46	1.53	1.46	0.07	0.89
Liver	8.08	8.58	7.58	8.7	8.7	0.43	0.30
Lungs	1.55	1.38	1.34	1.56	1.54	0.15	0.72
Heart	0.95	0.92	0.94	0.93	0.84	0.05	0.63
Spleen	0.63	0.64	0.70	0.68	0.60	0.04	0.46
GIT <sup>3</sup> (Full)	21	25.34	22.92	24.23	26.44	1.91	0.33
GIT (Empty)	16.93	17.84	16.9	16.10	19.76	1.45	0.47

\*SEM; <sup>1</sup>Cassava Root Meal; <sup>2</sup> Methionine; <sup>3</sup>Gastro-intestinal tract

**Table 4 - Effect of cassava root meal on blood parameters**

Parameters <sup>3</sup>	Control	30% CRM <sup>1</sup> 0.15% Met <sup>2</sup>	30% CRM 0.3% Met	45% CRM 0.15% Met	45% CRM 0.3% Met	SEM*	P-Value
WBC (x10 <sup>9</sup> /L)	10.62	10.90	8.28	10.34	11.92	1.711	0.589
RBC (x10 <sup>12</sup> /L)	7.14	7.22	7.15	6.84	7.21	0.264	0.829
HGB (mmol/L)	14.07	14.16	14.06	11.84	14.26	0.266	0.066
0.63HCT (L/L)	44.70	45.06	44.12	42.18	45.20	1.561	0.637
MCV (fL)	62.57	62.46	61.64	61.80	62.64	0.741	0.804
MCH (fmol)	19.72	19.64	19.64	17.78	19.80	1.068	0.619
MCHC (mmol/L)	31.52	31.44	31.92	28.64	31.58	1.647	0.607
PLT (x10 <sup>9</sup> /L)	899.50	940.60	842.4	975.0	940.0	125.71	0.951

SEM; <sup>1</sup>Cassava Root Meal; <sup>2</sup> Methionine; <sup>3</sup> WBC, White Blood Cells Count; RBC, Red Blood Cell Count; HGB, Haemoglobin; HCT, Haematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; PLT, Platelet Count.

The absence of this phenomenon in this trial indicates that although feed intake tended to increase with increasing levels of dietary CRM, it was not significant enough to cause any marked increase in GIT musculature. Cassava root meal contains cyanogenic glucosides, polyphenols (tannins) and phytate (Teguia and Beynen, 2004). These are poisonous when consumed by monogastrics in large quantities. Enlargement of certain internal organs like the pancreas, liver, and kidney have been linked to an excessive consumption of these anti-nutritive factors

(ANF) by broilers (Obun et al., 2011) and pigs (Adesehinwa et al., 2011). Methods to eliminate the ANF in cassava, particularly the cyanogenic glucosides, include drying and fermentation. The CRM used in this trial was sun-dried for 7 days. Again the variety of cassava that is grown in Ghana is of the low hydrocyanide variety. These two factors may have worked to ensure that the levels of the cyanogenic glucosides eaten by the rats were quite low and presented no health problems.

Hematological values obtained in this trial fell within the normal ranges of Sprague-Dawley rats (Johnson-Delaney, 1996; Adeyemi et al., 2015). This indicates that there was no effect of diet on haematological values.

## CONCLUSIONS

Results of this trial have demonstrated that CRM can effectively replace up to 70% of the maize in diets for Sprague-Dawley albino rats when supplemented with a synthetic amino acid like Met, without affecting growth performance. At the recommended level of inclusion, there are no adverse effects on internal organ and blood parameters.

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# COMPARISON BETWEEN THE PERCENTAGE OF INCIDENCE OF MASTITIS CAUSED BY *Bacillus* spp. AND *Staphylococcus* spp. IN WINTER SEASON IN KHARTOUM STATE, SUDAN

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**ABSTRACT:** This study was conducted in certain area at Khartoum State (Eltebna, Falasteen, Shambat, Hilat Kuku, Elhalfaia, Elsamrab and The University of Khartoum farms) in winter season to determine the type of mastitis and to compare between the incidence of mastitis caused by *Staphylococcus* spp and *Bacillus* spp. The total number of dairy cows, which were examined in 34 investigated farms, amounted to 500 animals, but the number of positive cows infected with mastitis were 100. The milk samples were collected from cows due to complain of owners from clinical cases of mastitis. Hundred milk samples were collected from apparent cases of mastitis. All mastitic cases were examined by visual examination and palpation of the udder: 55% acute mastitis, 44% chronic mastitis and 1% gangrenous mastitis were diagnosed. Milk samples were cultured in Blood agar and MacConkey's agar for 24 hours at 37° C. The isolation of *Bacillus* spp. amounted 74% , these constituted 31% *Bacillus coagulans*, 11% *B. cereus*, 9% *B. subtilis*, 9% *B. licheniformis*, 4% *B. circulans*, 2% *B. lentus*, 3% *B. mycoides*, 3% *B. amyloliquefaciens* and 2% *B. megaterium*. The percentages of acute mastitis caused by *B. coagulans* was 14%, *B. subtilis* 8%, *B. lichneformes* 7% and 2% for every followed *Bacillus* spp. (*B. cereus*, *B. circulans*, *B. lentus*, *B. mycoides*, *B. amyloliquefaciens* and *B. megaterium*). The percentage of chronic mastitis caused by *Bacillus* spp. were as follows: *B. coagulans* was 17%, *B. cereus* 9%, 2% for every *Bacillus* spp. (*B. lichneformes*, *B. circulans* and *B. lentus*) and 1% for every followed *Bacillus* spp. (*B. subtilis*, *B. mycoides*, *B. amyloliquefaciens* and *B. megaterium*). *Staph aureus* and *Staph hyicus* amounted to 24% and the percentage of chronic mastitis caused by *Staph aureus* was 44% and that caused by *Staph hyicus* was 8%. The percentage of acute mastitis caused by each species of *Staph* was the same 24%. Other bacteria were isolated from mastitic cows *Corynebacterium* spp. 1% and *Klebsiella* spp. 1% and the last one was isolated from gangrenous mastitis as first report in Sudan.

**Keywords:** Comparison, Incidence, Mastitis, *Bacillus* Spp., *Staphylococcus* Spp., Cattle, Winter, Khartoum, Sudan.

## INTRODUCTION

Mastitis is a multi-factorial disease and very difficult to control. It results from injury, chemical irritation and infection caused by different bacterial species. Mastitis is most expensive disease of dairy animals resulting in the reduction of milk production and quality. These expenses in terms of reduction of production, discarding milk, drug therapy, veterinarian charges, premature culling, and extra use of labour (Anonymous, 1998). Bovine mastitis is the inflammation of the parenchyma cells of the mammary glands of cattle, buffalo and other animals (Radostitis et al., 2007) associated with microbial infections (Schroeder, 1997) and physiological changes (Shouky et al., 1997). Mastitis is caused by a group of infective and potentially pathogenic bacteria (Bezek and Hull, 1995) viruses, fungi and algae (Radostitis et al., 2007).

The bacterial agents responsible to cause inflammation of udder are classified as either contagious or environmental, based upon their primary reservoir and mode of transmission. *Staphylococcus aureus* and *Streptococcus dysagalactiae* are recognized as contagious bacterial species, commonly transmitted among dairy animals through contact with infected milk, but the major pathogen for bovine mastitis is *Staphylococcus aureus* is regarded as being coagulase-positive, although some strains have been suggested in some studies to be coagulase-negative (Fox et al., 1996).

Some other *Staphylococcus* species may also be coagulase-positive (Hajek, 1976; Devriese et al., 1978 and Devriese et al., 2005). Some authors have suggested that infections with minor pathogens like *S. chromogenes* and *Corynebacterium* sp. could be beneficial as they might protect the quarter from mastitis caused by major pathogens such as *S. aureus* (Schukken et al., 1989 and Matthews et al., 1990).

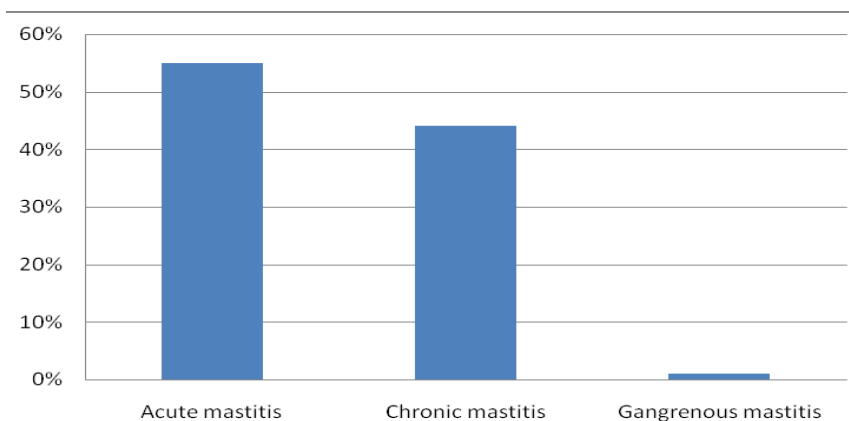
## MATERIAL AND METHODS

One hundred milk samples from clinical mastitis of cows were collected during 2008. Before collection of milk samples, the surroundings of teat canals were cleaned with antiseptics (spirit) and then first few drops of milk were discarded. The milk samples were collected in sterilized bijou bottles and brought to the Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Khartoum. The bacterial culture media were prepared and used for detailed investigation of bacterial organism by (Barrow and Feltham, 2003). Both, solid and broth media were used. In solid media: nutrient, blood and MacConkey's agars and while in broth medium: nutrient broth was prepared; cultured and colony characteristics were recognized.

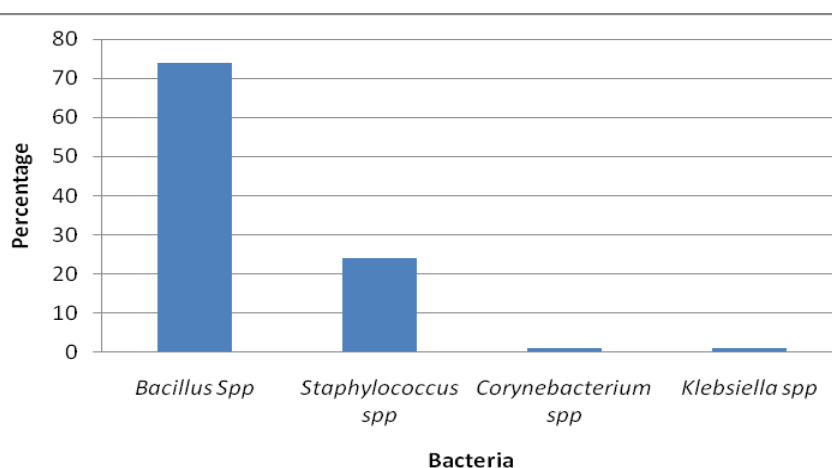
A pure colony from cultured dishes was picked up and smeared on a cleaned glass slide and stained by Gram's Method of staining and staining characteristics were recorded. Furthermore, a few biochemical tests were also conducted to confirm the identification of bacterial organisms, for this purpose, oxidase, catalase, coagulase, indole, oxidation fermentation, urease, methyl red, acid production from sugars (Barrow and Feltham, 2003).

## RESULTS

The five hundred samples which were examined and 100 cows were found positive for mastitis. Thirteen bacterial species were recognized from clinical mastitic milk samples of cows. The percentage type of mastitis and bacterial species identified from samples were: Percentage of different type of mastitis diagnosed during this study are shown in Figure 1 acute mastitis 55%, chronic mastitis 44% and gangrenous mastitis 1%. Figure 2 shows the percentage of isolated bacteria compared with *Bacillus* spp. The percentage of *Bacillus* spp. was 74%, *Staphylococcus* spp. 24%, *Corynebacterium* spp. 1% and *Klebsiella* spp. 1%. Figure 3 shows the percentage of isolated *Bacillus* spp. The highest one was *Bacillus coagulans* (31%). Figure 4 shows the percentage of acute and chronic mastitis caused by the isolated *Bacillus* spp. The percentage of acute mastitis caused by *Bacillus* spp. was higher than chronic mastitis. Figure 5 shows the percentage of *Staph* spp. and the type of mastitis caused by the isolated *Staph* spp. In this study the percentage of chronic mastitis caused by *Staph* spp. was higher than acute mastitis. The percentage of acute mastitis was found higher than chronic mastitis.

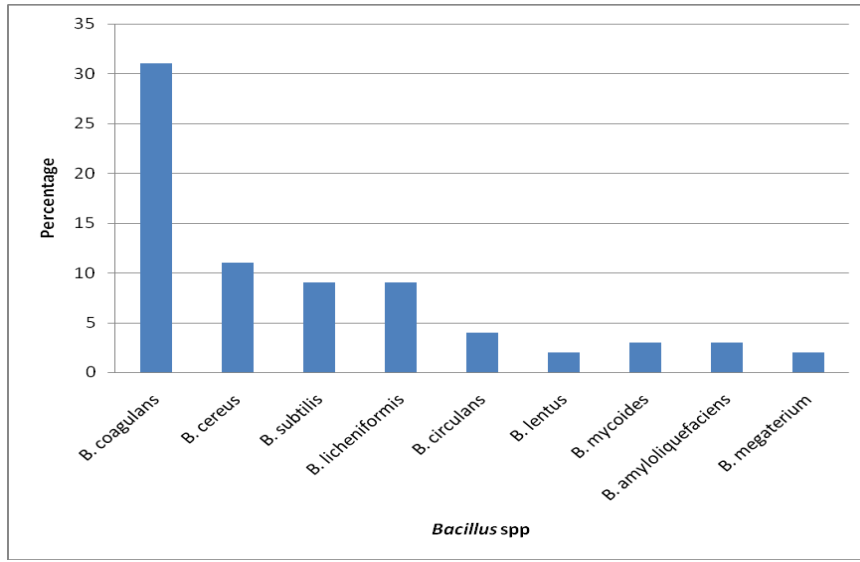


**Figure 1 - Classification of mastitis according to the clinical state of the mammary gland**



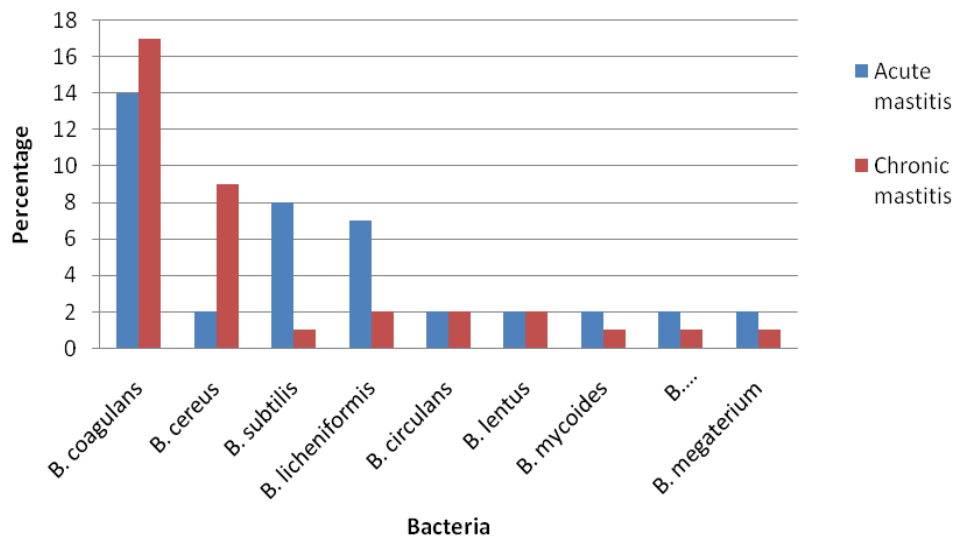
**Figure 2 - Percentage of isolated bacteria compared with *Bacillus* spp. Isolated from 100 mastitic cows**



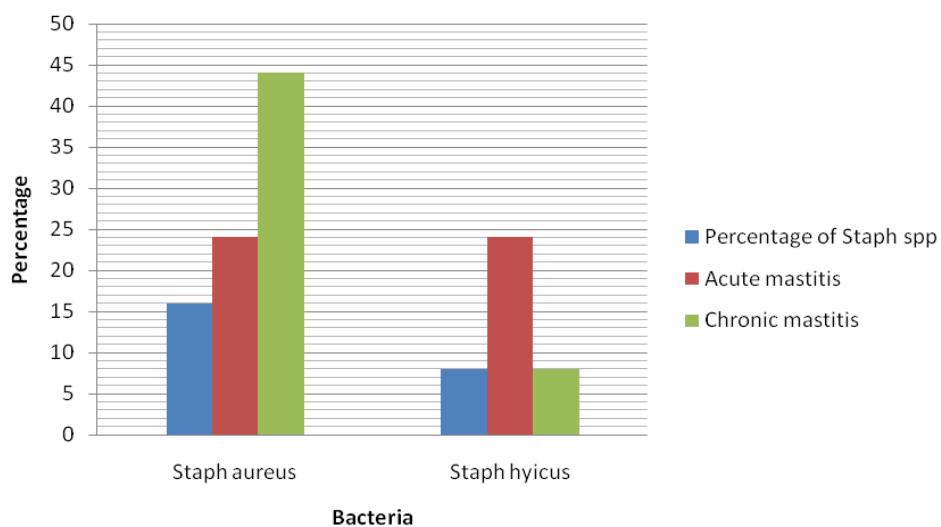


**Figure 3 - The percentage of isolated *Bacillus* spp. from 100 mastitic cows**

Percentage of type of mastitis caused by isolated *Bacillus* spp



**Figure 4 - Percentage of acute and chronic mastitis caused by isolated *Bacillus* spp. from 100 mastitic cows**



**Figure 5. The percentage of *Staph* spp. and the type of mastitis caused by the isolated *Staph* spp.**

## DISCUSSION

Mastitis is an inflammation of the mammary glands regardless of the cause (Blood et al., 1983). It plays a very important role in human health and animal (Kromber and Grabowski, 2002). In this study the types of *Staph* spp. isolated from acute and chronic mastitis were *Staph aureus* and *Staph hyicus*. This agrees with the finding of (DaRong et al., 2010 and Jan et al., 1998). (Radostits et al., 1994) mentioned that *Staph aureus* is the first microorganism incriminated in bovine mastitis. A predominance of *Staph aureus* mastitis in cows has been reported by (Watts, 1988; Falade et al., 1989 and Carlos, 1990). Elsayed, (2000) isolated *Staph aureus* and *Staph hyicus* from 499 milk samples from different domestic animals: cows, sheep, goat and camels. (AlAyies, 2004) isolated *Staph aureus* (73.7%) and *Staph hyicus* (6%) from 100 bovine mastitic milk samples. On conclusion this study confirmed the role of *Staphylococcus* spp. as a cause of mastitis in bovine. The percentage of incidence of bovine mastitis was high according to our findings after examinations.

*Bacillus* species are widely distributed in nature and most species exist in soil, water, dust, air, feces and on vegetation. The first case reported by Brown and Scherer (1957) was attributed to the introduction of the organism during treatment of chronic intra-mammary infections when a single plastic syringe was used by a dairy farmer to infuse the quarters with an antibiotic solution. In this study nine species of *Bacillus* were isolated from acute and chronic mastitis, this in agreement with (Jan et al., 1998). The percentage of incidence of *Bacillus coagulans* was high and this confirms the findings of (Nail et al., 2003). Also *Bacillus cereus* was also isolated by (Nail et al., 2003; Jasper et al., 1972 and Schiefer, 1976). Other species of *Bacillus* were isolated like, *B. licheniformes*, and this in accord with results of (Jones and Turnbull, 1981; Logan, 1988; Nail et al., 2003 and Parvanta, 2000). The isolation of *Bacillus alvei*, *B. subtilis*, *B. megaterium* and *B. cereus* during this study in agreement with (Elgadasi, 2003). *B. licheniformis*, *B. amyloliquefaciens*, *B. circulans*, *B. lentus* and *B. mycoides*, to the best of our knowledge for the first time to be recorded for mastitis in cow in Sudan.

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# AMELIORATION EFFECT OF PROBIOTICS (CHEESE) AND PREBIOTIC (GARLIC) ON AFLATOXIN B<sub>1</sub> INDUCED HEMATOLOGICAL ALTERATIONS IN FRESH WATER FISH *Cyprinus carpio* L.

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**ABSTRACT:** The objective of this study was to examine protective effect of probiotics against aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) on hematological and serum parameters which include the RBC, WBC, albumins, globulins, serum creatinine, and ALT and AST of fresh water fish *Cyprinus carpio* L. The total hemobiochemical analysis was compared with the AFB<sub>1</sub> induced and probiotics (garlic, cheese) treated groups. *Cyprinus carpio* L. (40 ± 10 g), were randomly divided into five experimental groups (15 fish per group). Group T1 represented the negative control fed with normal diet, and T2 was the positive control group fed with AFB<sub>1</sub> contaminated diet. Groups T3, T4 were fed with AFB<sub>1</sub>-contaminated diet 200ppb supplemented with 2 mg/kg cheese, 2 mg/kg garlic, and Group T5 fed with AFB<sub>1</sub>-contaminated diet and 4 mg/kg bw (garlic + cheese) probiotic supplementation in 1:1 ratio respectively. Ingestion of AFB<sub>1</sub>-contaminated fish feed possess the adverse effects on hematological parameters like, total red blood cells numbers, relative number of lymphocytes, monocytes, neutrophils, basophils, and eosinophils in blood. Likewise AFB<sub>1</sub> altered globulin, albumins, and total protein concentrations in serum fractions (GroupT2). Supplementation of probiotics cheese alone showed significant protective effect than garlic and in combination group (Group T5). Group 5 showed more or less similar to that of the control, in conclusion probiotics cheese and garlic showed significant combat effects in reducing hematological toxic effects of AFB<sub>1</sub>.

**Keywords:** Probiotics, Aflatoxin B<sub>1</sub>, Hematology, Cheese, Garlic.

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## INTRODUCTION

Aflatoxins are the toxic secondary metabolites produced by the fungal species *Aspergillus flavus* and *A. parasiticus* severely affects the fisheries through feed contamination. Among the different types of aflatoxins AFB<sub>1</sub> ranked 1<sup>st</sup> based on their toxic nature (Alinezhad et al., 2011). Hematological and serological techniques are the flagging evidences to screen the fishes by giving valuable information for fishery biologists in assessing the health of fishes and monitoring biochemical stress responses due to toxins and/or due to sub lethal concentration of pollutants (Zorriehzakra et al., 2010). Stress conditions influence blood parameters of fish (Bhaskar, 1983). A numerous hematological studies also clearly indicates that AFB<sub>1</sub> carcinogen toxin preliminarily effects the hematocrit (Sabbioni et al., 1990; Bakke et al., 1991), biochemical hematogram investigations mainly on cells present in the blood viz. red blood cells (RBC) count, hemoglobin concentration (Hb), which determines the functional status of oxygen carrying capacity of blood stream, packed cell volume (PCV), white blood cells (WBC) count, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV); whereas serology deals with the constituents in the fluid part of blood such as protein, enzymes, minerals, carbohydrates, pigments, hormones, immune bodies etc. (Rajeev et al., 2007). The serum proteins, composed of a non-homogeneous mixture, may be classified according to the various physical and chemical properties. Basically the serum proteins are divided into two major fractions albumin and globulins moreover some of the albumin and globulins are synthesized in the liver. The proteins in plasma and sera are chiefly involved in nutrition, water distribution, acid-base balance (Bhaskar, 1994), transport mechanism, immunity and enzymatic responses to specific metabolic needs. Serum protein concentrations can be used to monitor disease progress and general physiological status, as total protein levels tend to drop in diseased states (Bhaskar, 1994). Sequential total protein analyses provide quantitative evidence of disease progression (Searcy et al., 1964). Aflatoxin toxicity considerably depending on toxic concentration and exposure period in aquatic forms. Once absorbed into the blood, AFB<sub>1</sub> binds avidly to plasma proteins and loosely to red blood cells (Luthy et al., 1980; Kumagai et al., 1983). AFB<sub>1</sub> mainly bound to the serum albumins by forming adducts hydrolysis products of these epoxides reactive intermediate, aflatoxin B<sub>1</sub>-8, 9-epoxide can react with the ε-amino group of lysine in serum

albumin (AFB1-lys) (Sabbioni et al., 1990). This AFB1 serum albumin adducts used as hematological biochemical markers to detect the pathological status of liver cancers. The present study was under taken to finding the protective nature of probiotics cheese and garlic on total biochemical hematological changes in against AFB1 toxicity in *Cyprinus carpio* L.

## MATERIAL AND METHODS

### Fish acclimation

A total of 90 fishes of *Cyprinus carpio* L, ( $40 \pm 10$  g), were obtained from local fishery department at Tirupati, Andhra Pradesh, India. The fish were allowed to acclimate to their new glass housing aquarium conditions for 2 weeks before the start of the experiment. The water temperature, P<sup>H</sup>, dissolved oxygen and salinity was properly maintained daily. Food commercial basal diet was provided thrice daily (8 am, 2 pm and 6 pm) at the rate of 3% of the fish biomass. All water parameters were maintained within recommended ranges during the experimental period.

### Experimental design

All acclimated fishes were grouped totally in to 5 groups, Group T1 negative control treatment fish fed with basal diet, T2 positive control fish, fed a diet contaminated with 200 ppb AFB1, T3 fish were fed a diet contaminated with 200 ppb AFB1 + Cheese (2mg/kg bw), T4 Fish were fed a diet contaminated with 200 ppb AFB1+ Garlic (2mg/kg bw), T5 fish were fed a diet contaminated with 200 ppb AFB1 + Cheese + Garlic (2mg/kg bw).

### Chemicals

All the chemicals procured from the standard companies aflatoxin B1 was purchased from Himedia company and the pure AFB1 powder were placed at 2 °C and the powder was processed and extracted in methanol (1mg/ml) having the concentration 1001 ppm. For AFB1 determination, an aliquated volume of the pure extract was diluted in 10% methanol. The cheese and garlic was purchased from local market and made a crude extract of garlic (0.5mg/kg) the commercial cheese was procured from Amul Company (manufactured in Gujrat India code No: MMPO RC No.81/R-MMPO/93) which contain lactobacillus was given (0.5mg/kg) daily thrice through the commercial feed (Taiko manufactured in Chennai, India, code no: 01-1143).

### Blood collection

Fishes were anesthetized with 120 mg/l amino-benzoic acid (Sigma–Aldrich) before the drawing of blood. Fish blood collected from caudal vein using a disposable 1 cc tuberculin syringe and stored in two different vials one containing coagulant coted and immediately stored at -20°C, and another without the anticoagulant was kept at room temperature for about an hour.

### Separation of blood constituents

The partially clotted blood was kept inside the refrigerator for some time to ensure the complete shrinkage of the blood cells, which increased the yield of serum. Later the samples were subjected to centrifugation for ten minutes at 4000 rpm. Samples were used to determine the hemoglobin (Hb) content using a commercial kit (Cayman Chemical Item Number 700540), and the total erythrocyte (RBC) and leukocyte (WBC) counts using an hemocytometer (Neubauer improved) were obtained according to the methods described by Santiago Perez, other blood samples for serum separation were collected without the addition of anticoagulants and then centrifuged at 3000 g for 10 min. The activity of serum aspartate transferase (AST), alanine transferase (ALT) and creatinine was estimated according to the methods of Reitman (1957) and Frankel Luthy et al. (1980) and Henry (1974). The serum was collected carefully into small polypropylene tubes and stored at -20 °C until used for electrophoretic analysis. In addition, serum total protein, albumin and globulin were determined through SDS PAGE analysis.

### Total no of RBC Count

Total of RBC cells were counted by using the Neubaur hemocytometer and the blood was diluted with the 1:200 with Hayem's fluid, based on the location on chamber the total no of cells were counted and reported as  $10^6$  mm<sup>-3</sup> (Wintrobe 1967).

### Total no of WBC count

The total number of white blood cells were counted by using the Neubaur hemocytometer and the blood was diluting with 1:20 WBC diluting fluid and counted the number located cells on the 4 large corner squares under the microscope (Olympus) at 640X the total number of WBC were counted with  $\text{mm}^3 \times 10^3$ .

### Biochemical analysis

Percentage of hemoglobin was estimated with the commercial test kit (Hemoglobin Colorimetric Assay Kit, Cayman Chemical Item Number 700540), serum constituents creatinine with test kit (Liquizone Creatinine-MR, Medsource Ozone Biochemicals Pvt. Ltd, India) and Alanine transaminase (ALT) test with (Alanine Transaminase Activity Assay Kit, Cayman Chemical Item Number 700260), Aspartate transaminase (AST) tested using (Aspartate Aminotransferase (AST or SGOT) Activity Colorimetric Assay Kit, Life science source, Code: K753-100) the results were compared with the treated groups and the outcome results were conclude with statistical analysis by conducting student t-test. Protein analysis was done by Lowrey et al., (1954). And the serum protein factions were analyzed with standardized procedure SDS PAGE analysis Laemmli (1970).

## RESULTS

The results of hemo-biochemical analysis in all the groups T1, to T5 with serum and whole blood samples was as follows.

### RBC count

Oral supplementation of contaminated AFB1 to fresh water fish *Cyprinus carpio* L. significantly decreased the number of erythrocyte with a percent change of -54.38 when compared with control (Table 1). Fish treated with probiotics like cheese and garlic alone, groups T3 and T4 respectively, showed significant ( $P < 0.05$ ) increase in RBC count when compared to T2 group with a percent change of -11.84, -3.50 respectively (Table 1). And combined supplementation of cheese and garlic group T5 showed increase in the RBC number with percent change -10.52 and the migrating effect was greater even though the change was insignificant when compared with the T3 and T4 groups with the mean value of T2 (Table 1).

### WBC count

The white blood cells number in AFB1 contaminated feed receiving group (T2) showed a significant increase with percent change of 13.43 when compared with control (Table 1). Even though an increase in the mean value of WBC count was observed in T3, T4 and T5 groups with percent changes 8.12, 2.18 and 3.43 but the increase value was not significant (NS) (Table 1).

### Serum Analysis

**Albumins:** Albumin levels in T2 group (AFB1 treated group) decreased with percent change of -2.91 than that of control group but the reduction was insignificant (Table 1). In all probiotic treated groups T3, T4 and T5 elevated albumin levels were observed when compared with control group with a percent change of 13.14, 41.26 and 9.81 respectively, but this elevation was significant only in T3 group (AFB1+Cheese) (Table 1).

**Globulins:** Globulin levels were decreased significantly with percent change of -22.48 in T2 group (AFB1 treated) (Table 1). In all probiotic treated groups T3, T4 and T5 globulin levels were elevated significantly when compared with control group with a percentage change of 57.25, 64.90 and 47.31 respectively (Table 1).

**Cholesterol:** Cholesterol levels were decreased with percent change of -11.63 in T2 group (AFB1 treated) than that of control but the reduction was insignificant (Table 1). In all probiotic treated groups T3, T4 and T5 Cholesterol levels were significantly elevated when compared with control group with a percent change of 90.83, 120.13 and 65.43 respectively, but this elevation was significantly higher in T3 and T4 groups than that of T5 group (Table 1).

**Total Proteins:** Total protein levels were decreased significantly with percent change of -23.44 in T2 group (AFB1 treated) (Table 1). In all probiotic treated groups T3, T4 and T5 the total protein levels were similar to that of control group and the variation was non-significant with a percent change of -21.40, -0.26 and -6.064 respectively (Table 1).

**Aspartate Amino Transferase (AST):** Aspartate Amino Transferase (AST) levels were decreased significantly with percent change of -52.07 in T2 group (AFB1 treated) (Table 1). In all probiotic treated groups T3, T4 and T5 the AST levels were similar to that of control group and the variation was non-significant with a percent change of 8.41, -8.85 and 8.41 respectively (Table 1).

**Alanine Amino Transferase (ALT):** Alanine Amino Transferase (ALT) levels were increased significantly with percent change of 64.87 in T2 group (AFB1 treated) (Table 1). In all probiotic treated groups T3, T4 and T5 the ALT levels were similar to that of control group and the variation was non-significant with a percent change of 22.56, 4.61 and 11.96 respectively (Table 1).

**Creatinine:** Creatinine levels were increased significantly with percent change of 26.6 in T2 group (AFB1 treated) (Table 1). In all probiotic treated groups T3, T4 and T5 the creatinine levels were similar to that of control group and the variation was non-significant with a percent change of 1.6, 14.63 and 17.46 respectively (Table 1).



**Table 1.** Showing Mean  $\pm$  SD levels of albumin, hemoglobin, cholesterol, RBC, WBC, total protein, ALT, AST, creatinine and triglycerides in blood samples of control (T1), AFB1 treated (T2), AFB1 + garlic treated (T3), AFB1 + cheese treated (T4), AFB1 + garlic + cheese treated (T5) Fishes (*Cyprinus carpio* L). Values with in the brackets indicate in percentage change of that sample over the control.

S.NO		Control	AFB1	GARLIC + AFB1	CHEESE + AFB1	GAR + CHE + AFB1
1.	Hemoglobin	8.325 $\pm$ 0.56a	6.15 $\pm$ 0.47b (-26.44)	7 $\pm$ 0.46b (-16.22)	7.575 $\pm$ 0.55a (9.31)	6.375 $\pm$ 0.42b (-23.73)
2.	RBC	1.71 $\pm$ 0.10a	0.78 $\pm$ 0.10b (-54.38)	1.50 $\pm$ 0.13a (-11.84)	1.65 $\pm$ 0.04a (-3.50)	1.53 $\pm$ 0.08a (-10.52)
3.	WBC	8000 $\pm$ 365.14a	9075 $\pm$ 618.46b (13.43)	8650 $\pm$ 479.58a (8.12)	8175 $\pm$ 50a (2.18)	8275 $\pm$ 150a (3.43)
4.	Albumin	3.005 $\pm$ 0.48a	2.91 $\pm$ 0.50a (-2.91)	3.4 $\pm$ 0.43a (13.14)	4.24 $\pm$ 0.67b (41.26)	3.3 $\pm$ 0.27a (9.81)
5.	Globulins	7.5525 $\pm$ 0.56a	5.8525 $\pm$ 0.72b (-22.48)	11.8725 $\pm$ 0.59c (57.25)	12.45 $\pm$ 1.36c (64.90)	11.1225 $\pm$ 0.59c (47.31)
6.	Cholesterol	167.3 $\pm$ 17.36a	147.82 $\pm$ 15.09a (-11.63)	319.26 $\pm$ 21.98b (90.83)	368.27 $\pm$ 34.19b (120.13)	276.76 $\pm$ 35.30c (65.43)
7.	Total Proteins	10.43 $\pm$ 0.54a	7.98 $\pm$ 0.90b (-23.44)	8.19 $\pm$ 1.18a (-21.40)	10.40 $\pm$ 2.03a (-0.26)	9.79 $\pm$ 1.00a (-6.064)
8.	AST	0.9014 $\pm$ 0.13a	1.3702 $\pm$ 0.12b (52.07)	0.9768 $\pm$ 0.04a (8.41)	0.8212 $\pm$ 0.13a (-8.85)	0.9768 $\pm$ 0.04a (8.41)
9.	ALT	1.1704 $\pm$ 0.17a	1.929 $\pm$ 0.12b (64.87)	1.434 $\pm$ 0.19a (22.56)	1.224 $\pm$ 0.16a (4.61)	1.31 $\pm$ 0.12a (11.96)
10.	Creatinine	3.11 $\pm$ 0.42a	3.9375 $\pm$ 0.64b (26.6)	3.16 $\pm$ 0.48a (1.6)	3.565 $\pm$ 0.40a (14.63)	3.66 $\pm$ 0.25 (17.46)
11.	Triglycerides	148.3625 $\pm$ 18.53a	178.625 $\pm$ 17.04a (20.39)	160.2025 $\pm$ 27.18a (7.98)	129.0275 $\pm$ 18.93a (-13.03)	161.4525 $\pm$ 23.21a (8.82)

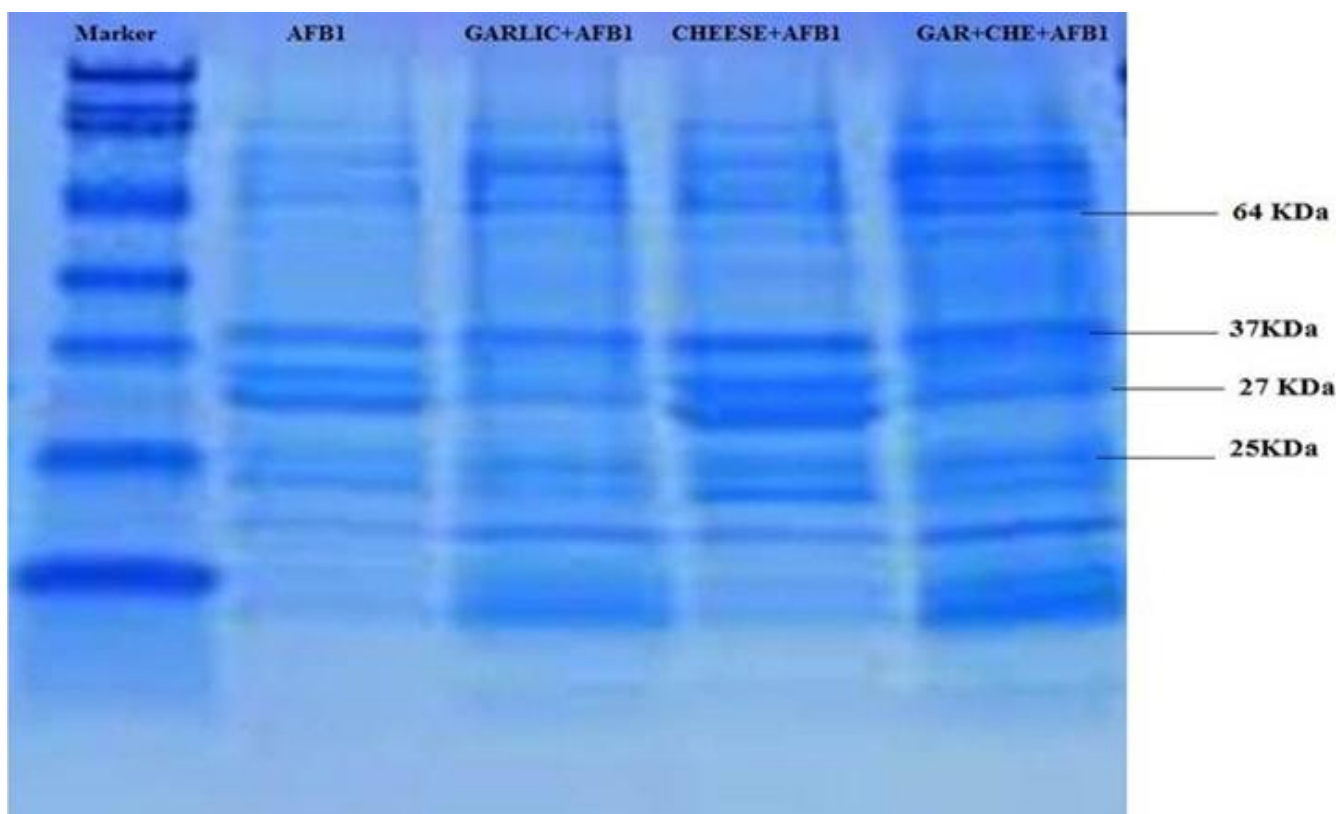
Values with same alphabetic superscript with in the row are not significantly different ( $p < 0.05$ )

### Triglycerides

Triglyceride levels were increased non-significantly with percent change of 20.39 in T2 group (AFB1 treated) (Table 1). In all probiotic treated groups T3, T4 and T5 the triglyceride levels were similar to that of control group and the variation was non-significant with a percent change of 7.98, -13.03 and 8.82 respectively (Table 1).

### SDS PAGE analysis of serum proteins

Serum proteins detected by SDS PAGE, only a few showed marked changes in their expression on exposure to aflatoxin B1. From the figure 1 it is evident that the general expressions of all the blood serum proteins above the molecular weight 97.4 KDa were weak in all the experimental groups, T4 and T5 were exposed for 21 days with aflatoxin in comparison with other groups (Figure 1). Their expression was however intensified in aflatoxin T2 group. Proteins of molecular weights 25 KDa, 29 KDa and 37KDa were decreased in the test groups T3 and T5 when compared with T2 and T4 groups (Figure 1). The AFB1 form adducts with the albumin proteins significantly and formed thick band with the molecular weight 64 KDa. The expression of this albumin adduct was very low in T2 group when compared with all other groups, in T5 group (AFB1+garlic treatment + cheese) the expression was high when compared with T3 and T4 groups (Figure 1).



**Figure 1.** Showing SDS PAGE analysis of serum protein in different groups of fishes (*Cyprinus carpio* L.). [Lane 1: molecular weight marker, Lane 2: AFB1 treated (T2), Lane 3: AFB1 + garlic treated (T3), Lane 4: AFB1 + cheese treated (T4), Lane 5: AFB1 + garlic + cheese treated (T5)]

### DISCUSSION

In the present investigation fishes fed with 200ppb AFB1 in oral diet altered blood parameters compared to that of control. AFB1 enters the blood streams and decreases the levels of RBC by suppressing the bone marrow cells. The mechanism of action by which aflatoxin aggravated progression of anemia could be attributed to down regulation of erythropoietin activity (Reddy et al., 1987). A lot of previous studies have been reported that AFB1 increased percentage of neutrophil counts and may cause lymphocytopenia and monocytopenia (Donmez et al., 2012). An increased WBC count (Table 1) suggests that aflatoxin B1 elicited an inflammatory response and cause alteration in bone marrow and enhances the production of WBC by immune system. Serum analysis of aflatoxin B1 treated fishes revealed a significant reduction in the total protein, globulin and albumin levels with increasing damage (Table 1). Similar observations were observed in Nile tilapia by Saber, (1995). The total protein levels were found to be decreased in the aflatoxin B1 fed farm animals and cockerels, but the protein expressions were elevated (Jacobs et al., 1994). The elevated levels of ALT, AST and are well-known sensitive marker enzymes of hepatic necrosis caused by the aflatoxins are well documented. In present investigation explore, aflatoxin B1

contaminated feed was found to cause a significant changes and enzymatic levels of ALT and AST of blood serum analysis indicators for the distractive normal metabolism of fish lead to liver carcinogenesis. Our results of decreased levels in above mentioned parameters were in agreement with Bhaskar et al. (1985) and Kececi (1998) also reported similar results on aflatoxin B1 induction Oguz et al. (2000) reported decreased hematocrit, hemoglobin levels, and MCV, erythrocyte, throm-bocyte, and lymphocyte counts. Furthermore, Basmacioglu et al., (2005) reported aflatoxin B1 induction to decrease in erythrocyte, lymphocyte, thrombocyte, MCV, hematocrit and hemoglobin levels counts in broilers. This decrease in the hematological parameters may be due to factors such as inhibition of protein synthesis due to lower serum albumin (Kaneko 1989) the hemopoietic cellular defects of aflatoxin B1 (Abdel-Wahhab et al., 2002; Van Vleet, 1992), or decrease of the total iron binding capacity (Harvey et al., 1991).

Our results also showed that there were considerable changes in serum creatinine levels. The elevated serum creatinine levels in groups T2, T3, T4, T5 indicate a toxic effect of AFB1 on the kidney, which was confirmed histologically (data not presented). Aflatoxin B1, which is a hepatotoxin in several fish species (Hendricks et al. 1993; Tuan et al., 2002), could have significantly changed the stability of the lysosomal membrane, leading to a hepatocyte permeability disorder and pathological changes in the liver of *Oreochromis mossambicus* (Varior and Philip, 2012).

This effect can be confirmed by high levels of ALT and AST enzymes in the blood. In the present study, we found significant increases in serum ALT and AST, confirming hepatotoxicity. Liver plays an important role in metabolism and excretion of AFB1 (Selim et al., 2014). It also plays an important role in detoxification or activation of toxic metabolites (Guengerich et al., 1998; Takahashi et al., 1995), Deng (2010) reported an AFB1- induced hepatic disorder in hybrid tilapia which was characterized by decreased hepatosomatic index, lipid content, and abnormal hepatic morphology. Aflatoxin adducts bind to cellular macromolecules leading to altered protein synthesis and loss of cellular integrity. This binding results in the reduction in total protein and albumin in serum (Patterson, 1976; Jindal et al., 1994; Abo-Norag et al., 1995). Our results also show the similar findings of decreased albumin and globulin levels in groups T2, T3, T4, T5 compared to negative controls of group T1. Yet, the levels in group T3 were comparatively better and almost similar to levels in negative control group T1. This could be due to the probiotic effect of cheese administered as part of treatment.

SDS PAGE analysis of serum proteins gives a brief picture of protein expression in AFB1 treated and probiotic treatment groups. The expression pattern as shown in the SDS PAGE more or less coincides with the biochemical analysis such as total protein albumin and globulin estimations (Table 1 and Figure 1). The AFB1 forms adducts with the albumin proteins significantly form thick band with the molecular weight 64 KDa. The serum albumin adducts in a dose dependent manner by binding to the lysine component of this protein, resulting in the formation of lysine – AFB1 which has been used to assess the level of exposure of aflatoxin B1 in humans (Sabbioni, 1990) which was clearly seen in our results (Figure 1). Aflatoxin B1 can also be converted to one of its metabolites, aflatoxin B2 that react readily with free amino groups of functional proteins. Aflatoxin B3 is not generally regarded as a mycotoxin and is believed to be in equilibrium with its dialdehyde, which reacts with the free amino groups to form schiffs bases, resulting in reduced enzyme activity (Moreau and Mass, 1979).

## CONCLUSION

Aflataxins are secondary metabolites of fungi that grow on a moisture feed and foodstuffs consumed by fishes. The present study evaluates the effect of probiotics such as cheese and garlic on toxicity of mycotoxin AFB1 on fishes. AFB1 consumption through contaminated diet decreased the hemogram status of blood constituents and serum markers by their membrane lysis nature and ready to form adducts with serum proteins and also decrease the total protein contents in blood. The results obtained clearly showed that probiotics cheese and garlic counteract the toxic effects of AFB1 by mitigating hemobiochemical parameters. Hence the combat supplementation of probiotic like cheese and garlic through feed may reduce the risk factors in fisheries and helps in improving the yield.

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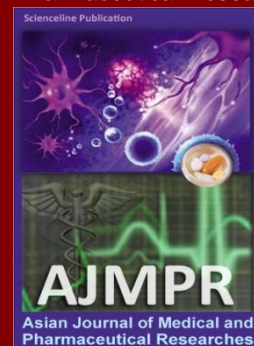
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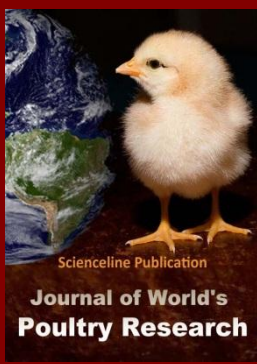
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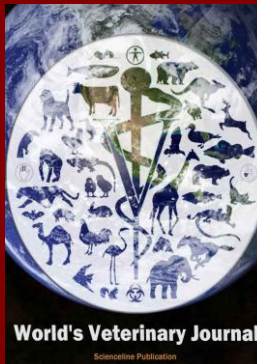
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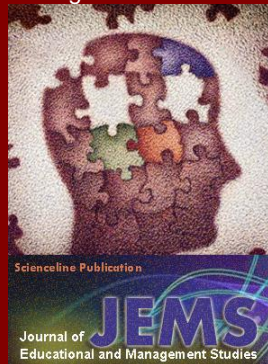
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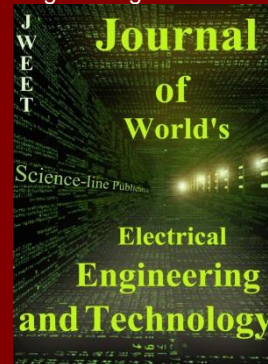
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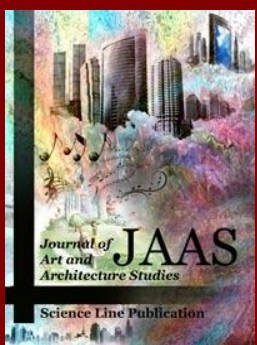
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