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COMPARATIVE ANALYSES OF MILK AND CHEESE SAMPLES OBTAINED FROM HOLSTEIN FRIESIAN AND BUNAJI COWS

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ABSTRACT

Microbiological and biochemical analyses of fresh milk samples collected form nine Holstein-Friesian and three Bunaji (White Fulani)cows under four different treatments (T_1 – T_4) at Maizube Farms, Minna, Niger State, Nigeria was carried out. Treatment T_1 comprised Bunaji breed, while treatments T_2 to T_4 comprised high, medium and low producing Holstein-Friesian breed, respectively. There were three replicates in each treatment to compare the quality of milk produced by the Bunaji and Holstein-Friesian breeds. Cheese was also produced from the different treatment milk samples to determine keeping quality. Biochemical analysis of the milk samples revealed crude protein, fat and moisture were not significantly ($p>0.05$) different but the values obtained for total solid and solid not fat were significantly ($p<0.05$) different. Milk yield was lowest in the Bunaji breed, the highest value being recorded in the high producing Holstein-Friesian breed (T_2). The least total bacteria count was recorded from T_4, being the low milk producing Holstein-Friesian group. This was followed by T_2 (high producers) and T_3 (medium producers), while the Bunaji (T_1) had the highest. Analysis of cheese samples revealed bacterial and fungal counts of between 0.00 – 7.62 x 10^7 ±0.59 x 10^7 and 0.00 – 4.24 x 10^7 ±0.66 x 10^7 CFU/g respectively. Various bacteria species identified included *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Tuberculosis bacilli*, while the yeast species identified on cheese samples were *Candida tropicalis*, *Candida torulopsis* and *Torulopsis dattila*. The results showed that milk yield of indigenous Bunaji cows was very low compared to the temperate breed. Bacteria count in milk samples obtained from local breed of cattle was higher than in milk samples obtained from the Holstein cows. However, no bacterial and fungal growths were observed on cheese samples obtained from the Bunaji breed. It is recommended that high sanitary measures should be observed to reduce contaminations in milk and cheese samples.

Keywords: Milk, Cheese, Bunaji, Holstein-Friesian, bacteria, fungi

INTRODUCTION

The problem of low milk yield and of low quality in dairy cattle under tropical environment is held as one of the most important limiting factors to for the growth of dairy industry in Nigeria. The problems have been attributed to a number of factors, among which are climate, genetic potentials of breeds, inadequate nutrition, disease and poor management standard (NLPD, 1992). In recent times, malnutrition has been recognized in developing countries of the tropics (Aduku and Olukosi, 1991). The measures adopted to correct these nutritional deficiencies in both child and adult populations in these countries are still debatable. There is however, almost complete unanimity of opinion on the fact that of all foods, milk and milk products play a leading role in the improvement of the nutrition of people, especially in areas where protein malnutrition is most prevalent (Cattle and Watkins, 1994).

Although more than half of the world's cattle population are found in the tropics, or in areas with similar climates, milk production from the vast cattle population account for only 15% of an estimated world total production of 400,932,000 metric tones. The remaining 85% come from cattle in the technically developed countries, most of which lie in the temperate latitudes especially in Europe and North America (CBN, 1999). In Nigeria, milk production is almost entirely from range cattle of Fulani pastoralists. However, the milk they produce is of low yield and poor quality. Unsanitary methods of milking, low genetic potential of dairy breed and generally poor managerial condition (FAO, 1990) and reduce milk products for the consumers have been identified in this group. However, the high price of imported processed milk products seems to have made consumers more ready to accept the locally produced milk products (Aduku and Olukosi, 1991).

The quality of milk produced by the tropical livestock has continually been a source of concern to research and government institutions (Voh Jr. and Otchere, 1989). Several attempts have been made to ensure improvement in the quality and quantity of milk obtained from livestock but the results obtained have not been commensurate with the enthusiasm (Aduku and Olukosi, 1991). Therefore, there is still need to improve the level and quality of milk production in Nigeria. This could be done by the importation of exotic breeds that yield more milk and produce better quality products than the local breed.

The objective of the present study is to compare the milk yield and keeping quality of cheese as well as the biochemical and microbial qualities of milk samples obtained from Holstein-Friesian and Bunaji (White Fulani) cows at a farm in Minna, Central Nigeria.

MATERIALS AND METHODS

Location of the experiment: The experiment was conducted at Maizube Farm, which is located 26 km from Minna Township, along Minna-Bida-Sabon-dagah road. Minna is located within latitudes 09^{0}30' and 0945' N and longitudes 06^{0}30' and 06^{0}45' E. It has an attitude of 1475 meters above sea level and is bounded by River Niger running the North Western flank down to the South Western part of the state. It falls within the southern guinea savanna agro ecological zone of the country. The mean annual rainfall varies from 1102.6 to 1361.7mm and annual temperature of between 26.66 to 27.77^{0}C (NSADP, 1995).

Animals and their management: The animals used for the experiments were twelve in number, (nine exotic Holstein-Friesian and three Bunaji local breed). The management system was purely intensive for the Holstein-Friesian and semi-intensive (natural grazing and feed supplements) for the Bunaji. Generally, the Holstein-Friesian cows were fed exclusively on total mixed ration (TMR) ad-libitum and were strictly not allowed out for grazing. They were routinely vaccinated against diseases, regularly sprayed against ecto-parasites and dewormed. Artificial Insemination (AI) was duly practiced.

Milk sampling: Milk samples were collected during six weeks from the experimental cows early in the morning between 6.30 and 9.00 am and in the evening from 4.00 to 6.00 pm, every Tuesday and Thursday. Fresh milk samples (12/collection) were collected in properly labeled 60 ml sterile bottles from both breeds. They were stored immediately in an ice packed cooler before transferring to freezer at 0^{0}C until required for analysis.

The animals (Friesians) were walked from their beddings through the passage to the waiting area where they were cooled with teat wash and cooling sprays. They were moved into the milking parlor, with capacity of 16 cows per milking. Nine cows were selected for milk sampling in three (3) groups; the high yielding (T_2), the medium yielding (T_3) and the low yielding (T_4). The cows were identified through the use of mini-computers in the milking parlor.

Teat cups were attached to the udders of the animals and milk was secreted by the action of air pressure. The three Bunaji cows (T_1), were subjected to hand milking, which was carried out by the Fulani pastoralists who reared the animals.

Cheese: Cheese was produced from the milk obtained from both breeds of Holstein-Friesian and Bunaji cows. The four cheese samples were produced at the first week of milk sample collection and were again distributed into four treatments: T_1, T_2, T_3, T_4 in four plastic cups.

After production, the samples were kept covered on the laboratory table, under ambient temperature for three days and later refrigerated for six weeks. Samples were taken from each treatment at the middle of every week for microbial analysis.

Parameters measured:

1. Microbial analysis of milk samples obtained from Holstein-Friesian and Bunaji cows.
2. Chemical composition of milk samples.
3. Quantity of milk/milk yield from individual replicates during the experimental period.
4. The keeping quality of cheese made from both Bunaji and Friesian cow's milk.

Analytical procedure: Microbial assessment of the milk samples was carried out for bacterial and yeast contents using standard plate count test (APHA, 1980). The cheese samples were also assessed for bacteriological quality using the standard plate count.

Biochemical analyses were carried out according to the AOAC (1995) procedures. The milk samples were analyzed for total solid (TS), crude protein (CP), fat, solid non-fat (SNF) and moisture.

The average daily milk yield for each replicate in treatments T_2, T_3 and T_4 were obtained through the computer records in the farm, while for the control (T_1), milk samples had to be quantified through the use of measuring cylinder in the animal production laboratory.

Statistical analysis: All data on milk samples were pooled and subjected to one-way analysis of variance (ANOVA) and test of significant difference was done using the Statistical Package for Social Scientists (SPSS) version 10 for window, 2002.

RESULTS AND DISCUSSION

Chemical composition of milk: The mean value (%) of crude protein, total solid, fat and solid non fat of milk obtained from Bunaji and Holstein-Friesian cows were summarized in table 1. Moisture, crude protein and fat did not differ significantly ($p>0.05$) among the different treatments, however values obtained for total solid and solid non fat differed significantly ($p<0.05$).
Although better values were obtained in the high and low milk producing Holstein cows (T_2 and T_4), Bunaji cows (T_1) exhibited a significantly ($p<0.05$) better performance than the medium milk producing Holstein-Friesian cows. This result could be attributed to the type of diet fed to the different breeds of cows. Holstein-Friesian cows, which were managed intensively on total mixed ration (TMR) produced larger volume of milk richer in total solid and solid non fat. The differences observed in the performance of the high, medium and low producing Holstein-Friesian was in line with the observation of Payne (1990) that differences could exist between cows of the same breed in their production potential and that milk composition could vary between one milking and the next. The extremely high (T_2) and extremely low (T_4) volume of milk could influence the concentration of milk hence the slightly better performance of Bunaji (T_1) over the medium producing Holstein-Friesian cows (T_3).

Table 1: Chemical composition of milk collected from Bunaji and Holstein Friesian cows at Maizube Farm, Minna, Niger State, Nigeria.

	Treatments				
Parameter	T_1	T_2	T_3	T_4	Level of Significance
Moisture	89.04±0.06	88.80±0.13	89.27±0.11	88.00±0.09	NS
Crude Protein	3.18±0.03	3.27±0.02	3.28±0.02	3.37±0.01	NS
Total Solid	10.96 ± 0.06^{bc}	11.20 ± 0.13^{b}	10.73 ± 0.11^{c}	12.00 ± 0.09^{a}	*
Fat	4.47±0.09	4.71±0.07	4.73±0.05	4.48±0.08	NS
Solid not fat	6.52 ± 0.11^{b}	6.47 ± 0.13^{b}	6.06 ± 0.12^{c}	7.52 ± 0.08^{a}	*

abc = Mean on the same horizontal rows bearing different superscripts are significantly ($p<0.05$) different
T_1 = Bunaji cow
T_2 = High milk producer (Holstein cow)
T_3 = Medium milk producer (Holstein cow)
T_4 = Low milk producer (Holstein cow)
* = Significant ($P<0.05$)
NS – Not significant ($P>0.05$)

Average daily milk yield: A significant difference ($p<0.05$) existed between the mean average daily milk yield obtained from Bunaji (T_1) cows and the Holstein Friesian cows ($T_2 - T_4$). The range of 0.94 to 1.10 litres of milk yield for the control (T_1) is very low compared to other treatments. This is due largely to the genetic potential of both breeds of cattle (Mabbett, 1991). The different systems of management coupled with the different plane of nutrition also have contributory roles to play. The management system was purely intensive for the Holstein Friesian breed fed exclusive total mixed ration (TMR) *ad-libitum*. Semi-intensive system was applied to the Bunaji breed fed with feed supplements after grazing naturally on pasture.
There was a lot of variation among the Holstein-Friesian cows milk yield, which ranged from 13.50±1.23 to 15.83 ±0.94 (T_4), 19.67 ± 1.17 to 23.33 ± 0.42 (T_3) and 25.67± 0.88 to 31.67 ± 1.98 for the high producers (T_2). Since most of them are first milkers and still growing, their anatomical and physiological machineries necessary for milk production and secretion are not yet fully developed (Sing and Tommar, 1991). This is responsible for the result obtained for the T_4 group, but as the cow matures from group T_3 to T_2, the structures for milk production and secretion develops too, thereby yielding more milk.
Microbial counts of milk samples: The number of bacteria present at the time of milking has been reported to range between several hundreds and several thousands milliliter (APHA, 1980). The bacterial count (Table 3) in fresh milk samples varies significantly ($p<0.05$), the highest been recorded in T_1. This could be attributed to the fact that milk samples were drawn under dirty

conditions. The sources of bacteria in the milk could be via the cow's hair, dung, feedstuff, air borne or from the soil, milk handling equipment and the milkers' clothes, body or hair.

Table 2: Average daily milk yield (liters) obtained from Bunaji cows (T1) and Holstein-Friesian cows at Maizube Farm, Minna, Niger State, Nigeria.
N = 288

	Treatments				
Parameter Week	T_1	T_2	T_3	T_4	Level of Significance
1	1.07 ± 0.03^d	31.67 ± 1.98^a	23.33 ± 0.42^b	15.83 ± 0.95^c	*
2	1.03 ± 0.02^d	28.83 ± 0.87^a	22.17 ± 0.70^b	$15.33\pm1{:}52^c$	*
3	0.96 ± 3.31^d	26.83 ± 0.95^a	21.17 ± 0.87^b	13.67 ± 1.38^c	*
4	0.93 ± 0.04^d	26.83 ± 0.79^a	21.83 ± 0.60^b	13.50 ± 1.23^c	*
5	0.90 ± 0.04^d	25.67 ± 0.88^a	19.67 ± 1.17^b	14.17 ± 1.20^c	*
6	0.90 ± 0.07^d	26.83 ± 0.79^a	22.50 ± 0.79^b	14.67 ± 1.41^c	*

abc = Mean on the same horizontal rows bearing different superscripts are significantly ($P<0.05$) different
T_1-T_4 = Treatment one to treatment four
± = Standard error of mean
* = Significant ($p<0.05$)
n = Total quantity of daily milk yield recorded

Shehu *et al.* (1998) and Goji (1984) confirmed that handling practices and level of hygiene of container used for milk collection influenced the increasing plate count. The Holstein-Friesians had lower plate counts due to the modern milking method. The multiplication of bacteria is influenced by the degree of bacterial contamination in the milk. Bacterial growth is less in clean milk as indicated by T_2, T_3 and T_4 milk samples in which considerable portion of the organisms came from the interior udder and less from external sources.

Table 3: Weekly comparative study of total viable bacterial count (CFU/ml) in milk collected from Bunaji cows (T1) and Holstein-Friesian cows at Maizube Farm, Minna, Niger State, Nigeria

	Treatments				
Week	T_1	T_2	T_3	T_4	Level of Significance
1	$0.98\times10^7\pm0.85$x1	$0.80\times10^7\pm0.06$x	$1.32\times10^7\pm0.14$x	$0.13\times10^7\pm0.065$	*
2	$1.02\times10^7\pm0.07$x1	$0.88\times10^7\pm0.04$x	$1.55\times10^7\pm0.13$x	0.00 ± 0.00^c	*
3	$1.12\times10^7\pm0.08$x1	$0.91\times10^7\pm0.05$x	$1.49\times10^7\pm0.44$x	$0.05\times10^7\pm0.03$x	*
4	$0.97\times10^7\pm0.09$x1	$0.96\times10^7\pm0.06$x	$1.37\times10^7\pm0.20$x	$0.01\times10^7\pm0.01$x	*
5	$1.08\times10^7\pm0.08$x1	$1.00\times10^7\pm0.04$x	$1.61\times10^7\pm0.10$x	$0.04\times10^7\pm0.02$x	*
6	$2.05\times10^7\pm1.08$x1	$1.08\times10^7\pm0.08$x	$1.84\times10^7\pm0.11$x	0.00 ± 0.00^c	*

CFU/ml Colony forming units per milliliter of milk sample

Microbial and fungal counts of cheese samples: The analysis indicate that T_1 (Bunaji) had the least bacteria and fungal count of 0.00 (Table 4). The highest contamination using both nutrient agar (NA) and Sabouraud dextrose agar was observed in T_2, then T_3 and T_4, respectively, in decreasing order.

There was a significant difference ($p<0.05$) in the results obtained due to the variation in the system of management for the two breeds of cows used for this study. The Bunaji cows, which were managed semi-intensively, had access to graze on natural pasture. The pasture grass contains carotene, which is naturally an anti-oxidant that is able to control oxidation. The milk produced by the Bunaji consequently is rich in carotene, which prevents rancidity from occurring, thus reducing activities of micro-organisms in the cheese samples obtained form Bunaji cows. The intensively managed Holstein Friesian were fed on exclusive total mixed ration (TMR). Hay, which is one of the feedstuff, offered to them is processed and prescribed for a long time, in the process losing its anti-oxidative quality. Therefore, the cheese produced from the Bunaji cows milk has better keeping quality and longer shelf life

Table 4: Weekly comparative study of total bacteria and fungal counts of cheese samples (CFU/g) produced form Bunaji cows and Holstein Friesian cows milk wit the use of NA and SDA at Maizube farms Minna, Niger state, Nigeria

Count	T_1	T_2	T_3	T_4	Level of significance
Bacterial	0.00 ± 0.00^{7}	$7.62 \times 10^{7} \pm 0.59 \times 10^{7}$	$7.34 \times 10^{7} \pm 0.57 \times 10^{7}$	$5.10 \times 10^{7} \pm 0.34 \times 10^{7}$	*
Fungal	0.00 ± 0.00^{7}	$4.24 \times 10^{7} \pm 0.66 \times 10^{7}$	$4.11 \times 10^{7} \pm 0.62 \times 10^{7}$	$2.62 \times 10^{7} \pm 0.50 \times 10^{7}$	*

NA = Nutrient Agar
SDA = Sabouraud Dextrose Agar
CFU/g = Colony forming units per gram of cheese sample
* = Significant ($P < 0.05$)

Identification of microbial isolates from milk samples: The isolation of *Staphylococcus aureus, Streptococcus pneumoniea, Tuberculosis bacilli and Bacillus subtilis* in this study (Table 5) is similar to that observed in earlier work by Adesiyun (1995), Shehu *et al.* (1998) and Abdulrahman (2004). The predominant bacteria revealed in this study was *Staphylococcus aureus,* no coliform was detected, while tuberculosis bacilli was detected in one of the high milk yielding producers.

Table 5: Characterization and identification of bacteria isolated from Bunaji cow's milk (T_1) and Friesian cow's milk (T_2, T_3, T_4) at Maizube Farm, Minna, Niger State

Sample	Grams reaction	Catalase test	Coagulase test	Starch hydrolysis test	Hydrogen sulphide	Methyl red test	Voges proskauer test	Oxidase test	Motility test	Indole test	Carbohydrate utilization: Sucrose	Fructose	Mannitol	Lactose	Glucose	Organism
T_1	+C	+	+	-	-	-	-	-	-	-	+	+	+	-	+	*Staph. aureus*
	+C	-	-	-	-	-	-	-	-	-	+	+	-	-	-	*Strep. pneumonia*
T_2	+C	+	+	-	-	-	-	-	-	-	+	+	+	-	+	*Staph. aureus*
	-R	-	-	+	-	-	+	-	+	-	+	+	+	+	+	*T. bacilli*
	+C	-	-	-	-	-	-	-	-	-	+	+	-	-	+	*Strep. aureus*
T_3	+C	+	+	-	-	-	-	-	-	-	+	+	+	-	+	*Staph. aureus*
	+R	+	-	+	-	-	+	-	+	-	+	+	-	-	+	*B. subtilis*
T_4	+R	+	-	+	-	-	+	-	+	-	+	+	-		+	*B. subtilis*
	+C	+	+	-	-	-	-	-	-	-	+	+	+	-	+	*Staph. aureus*
	+C	-	-	-	-	-	-	-	-	-	+	+	-	-	+	*Strep. pneumoniea*

+R = Positive rod shape
-R = Negative rod shape
+C = Positive cocci shape
+ = Positive result
- = Negative result

S. aureus have been reported as one of the most important cause of food poisoning associated with milk products (Adesiyun, 1995). Coagulate and hemolysin produced by the organism cause coagulation and hemolysis of blood cells (Umoh *et al.*, 1991). *S. Aureus* has also been proved as the aetiologic agent of bovine, caprine and ovine mastitis (Blood and Radostitis, 1983).

The presence of *Bacillus spp.* in milk samples could be due to their ability to form spores, which are capable of withstanding harsh environmental conditions and contaminate available materials. Another possible source of pathogenic bacteria in milk is through infected udder.

Identification of bacteria isolates from cheese samples: Bacteria which occurred in the cheese samples were identified as *Staphylococcus aureus and Bacillus subtilis* (Table 6). They occurred in all the treatments (T_2, T_3 and T_4) samples, while the control (T_1) had none. The bacterial isolates listed in this study are suspected to contaminate the samples from various sources as a result of poor handling and storage after collection of milk but most importantly, the absence of contaminants in the T_1 samples is directly related to the type of feed given to the Bunaji cows.

Table 6: Characterization and identification of bacteria isolated from Cheese produced from Bunaji cow's milk (T_1) and Holstein-Friesian cow's milk (T_2, T_3 and T_4) milk at Maizube Farm, Minna, Niger State

Sample	Grams reaction	Catalase test	Coagulase test	Starch hycrolysis test	Hydrogen sulphide test	Methyl red test	Voges proskaucer test	Oxidase test	Motility test	Indole test	Carbohydrate utilization: Sucrose	Fructose	Mannitol	Lactose	Glucose	Organism
T_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
T_2	+C	+	+	-	-	-	-	-	-	-	+	+	+	-	+	*Staph. aureus*
	+R	-	-	+	-	-	+	-	+	-	+	+	-	-	+	*Bacillus subtilis*
T_3	+C	+	+	-	-	-	-	-	-	-	+	+	+	-	+	*Staph. aureus*
	+R	+	-	+	-	-	+	-	+	-	+	+	-	-	+	*Bacillus subtilis*
T_4	+C	+	+	-	-	-	-	-	-	-	+	+	+	-	+	*Staph. aureus*
	+R	+	-	+	-	-	+	-	+	-	+	+	-	-	+	*Bacillus subtilis*

+R = Positive rod shape
+C = Positive docci shape
-R = Negative rod shape
+ = Positive result
- = Negative result

Identification of fungi (yeast species) on cheese samples: Table 7 showed that there was no fungal growth in T_1 (control) samples, but T2 and T3 contained pathogenic yeast of the class Blastomycete (*Candida tropicals* and *Candida torulopsis)* as the yeast occurring most frequently. The predominant yeast occurring in T_4 was *Torulopsis dattila*. The *Candida species* found were pathogenic and can cause disease, which might be systemic, cutaneous and mucocutaneous. These fungi are causative organisms of candidiasis. Candidiasis affects a wide variety of systems. Common examples of this are vaginitis, vulvar rash, oral thrush, conjunctivitis, endophthalmitis, diaper rash in children and infection of the nail, rectum and other skin folds.

Table 7: Identification of fungi (yeast species) in cheese samples

Sample	Macro-culture (Colonial appearance)	Hyphae	Spore	Organism
T_1	NIL	NIL	NIL	NIL
T_2	Initially flat to raised colonies. Initial colour is grey yellow as it is aging in more than 24 hours incubation the colour becomes only pure grey colour	Septate	No spore	Candida tropicalis
T_3	The colony is semi glossy initially and as it ages, it changes to smooth rippled. Initial colour is grey after 24hours it changes to white.	Non-septate (No hypae)	Spore	Candida torulopsis
T_4	The colonies are demarcated and are round, smooth in shape. The colour is cream throughout	Non-septate (No hypae	Spore.	Torulopsis dattila

CONCLUSION AND RECOMMENDATION

From the results obtained, it was concluded that a lot of variation existed in the total solids and solid not fat of milk, milk yield, population of microbial count in both milk and cheese samples between the Bunaji breed and Holstein-Friesian breed and even within the temperate breed.
In view of this, high sanitary practices during collection, processing and storage of fresh milk should be maintained, especially in the semi-intensive system. Similarly, proper veterinary attention should be paid to all animals to prevent transmission of harmful diseases such as tuberculosis. It is also recommended that cheese should be produced from Bunaji cow's milk for longer shelf life and better quality.

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