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# Veterinary Science and Veterinary Medicine

Glossina Pallidipes

Austen and Glossina

**Highlights**

Trypanosoma Brucei

Fuscipes Fuscipes Newsteed

Discovering Thoughts, Inventing Future

VOLUME 14 ISSUE 1 VERSION 1.0

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# Study on Odour Attractants to Catch *Glossina pallidipes* Austen *and Glossina fuscipes fuscipes* Newstead (Diptera: Glossinidae) at Sor Hydroelectric Station, Ethiopia

By Waktole Terfa, Abebe Olani & Firaol Tamiru
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*Abstract-* An experimental study was conducted to assess effects of odour attractants on catch size of Glossina pallidipes Austen and Glossina fuscipes fuscipes Newsteed at Sor Hydroelectric station around Sor River, West Ethiopia. Various doses of acetone, octenol and cow urine were used as odour attractants with monopyramidal traps. Three 5x5 Latin squares method was used making a total of 15 replicates. Acetone released at dose rate of 150mg/hr and 500mg/hr with 0.5mg/hr octenol and 1000mg/hr cow urine were found to be most effective in increasing catch size of Glossina pallidipes Austen by up to 11.26-12.54 times. Acetone released at dose rate of 2000mg/hr in combination with 0.5mg/hr octenol increased the catch only up to 4.07 times but cow urine alone produced increases in catch size by 2.54 times.

*Keywords:* *Glossina fuscipes fuscipes* Newstead*, Glossina pallidipes Austen, Odour Attractant, Sor River.*

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# Study on Odour Attractants to Catch *Glossina pallidipes* Austen *and Glossina fuscipes fuscipes* Newstead (Diptera: Glossinidae) at Sor Hydroelectric Station, Ethiopia

Waktole Terfa [α], Abebe Olani [σ] & Firaol Tamiru [ρ]

*Abstract-* An experimental study was conducted to assess effects of odour attractants on catch size of *Glossina pallidipes* Austen and *Glossina fuscipes fuscipes* Newsteed at Sor Hydroelectric station around Sor River, West Ethiopia. Various doses of acetone, octenol and cow urine were used as odour attractants with monopyramidal traps. Three 5x5 Latin squares method was used making a total of 15 replicates. Acetone released at dose rate of 150mg/hr and 500mg/hr with 0.5mg/hr octenol and 1000mg/hr cow urine were found to be most effective in increasing catch size of *Glossina pallidipes* Austen by up to 11.26-12.54 times. Acetone released at dose rate of 2000mg/hr in combination with 0.5mg/hr octenol increased the catch only up to 4.07 times but cow urine alone produced increases in catch size by 2.54 times. In the trial performed for *Glossina fuscipes fuscipes* Newstead, 0.5mg/hr octenol, 500mg/hr acetone and 1000mg/hr cow urine were used alone and in combination, the catch number of samples from baited and unbaited (control) traps differed slightly. There were no significant repellent or attraction effect as the efficiencies were at roughly the no odour level. In conclusion, cow urine (1000mg/hr) with octenol (0.5mg/hr) and acetone (150 and 500mg/hr) was considered to be a potentially useful combination of baits for *Glossina pallidipes* Austen control and sampling. However, further study should be conducted on behavior and baites used to attract *Glossina fuscipes fuscipes* Newstead in the study area.

*Keywords: Glossina fuscipes fuscipes* Newstead, *Glossina pallidipes* Austen, *Odour Attractant, Sor River.*

## I. Introduction

Trypanosomosis, a disease caused by protozoan parasites of the genus *Trypanosoma*, is still the main constraint to livestock production in Africa, preventing full use of land to feed the rapidly increasing human population (Leak, 1999). The disease is transmitted cyclically by tsetse flies that infest 37 African countries and mechanically by biting flies (Uilenberg, 1998). It has a devastating effect on livestock and man (IAEA, 2003) and can be controlled by drugs or by breaking the transmission cycle, which requires control of the vector tsetse flies. One of the important developments in tsetse control is identification and synthesis of odour attractants for some tsetse species, which greatly increases the efficacy of traps and targets (Leak, 1999).

Tsetse flies use the odours of their vertebrate hosts as cues in host location (Vale, 1974a; FAO, 1992). Some of the active components of breath, urine and skin secretion of hosts have been isolated for use at odour-baited targets and traps used to control and monitor tsetse populations (Dransfield *et al.*, 1986; Vale *et al.*, 1986; Leak, 1999). Acetone, 1-octen-3-ol (octenol) and carbon dioxide from bovine breath and phenols from urine are potent olfactory attractants for several species of the *morsitans* group of tsetse (Vale and Hall, 1985a; Hassanali *et al.*, 1986; Bursell *et al.*, 1988). However, such advances in catch size of some species the *palpalis* or *fusca* group of tsetse has not been realized as much as needed.

Several effective odours are known to attract *Glossina pallidipes* (G. *pallidipes)* (FAO, 1992) including odours of host animals and their residues, which are highly attractants for *G. pallidipes* Austen (Vale, 1974b; Hargrove and Vale, 1978; Vale and Hall, 1985a; Dransfield *et al.*, 1986). Electroantennogram studies in laboratory showed that both sexes of *Glossina fuscipes fuscipes* (*G. f. fuscipes*) Newstead were stimulated by components of bovine odour such as acetone, octenol, 4-heptanone and 3-nonanone (Den Otter *et al.*, 1988). In contrast, Mwangelwa *et al.* (1990) did not observe any consistent effect on catches of *G. f. fuscipes* in biconical traps baited with acetone, octenol, cow urine, phenols or monitor lizard wash.

The success of odour-baited devices for survey and control of *G. pallidipes* in other countries prompted studies in Ethiopia on the attractiveness of natural and synthetic odours, prior to their application in control operations. Thus, more information is needed on the dose response relationships for such attractants, as well as on their effects on the catch of *G. f. fuscipes*. Although baited traps and targets also offer great potential for control or eradication of tsetse flies (Vale, 1980), baseline data should be generated on odour

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attractants like cow urine, acetone and octenol in Ethiopia. Therefore, this study was conducted to assess effects of odour attractants on catch size of *G. pallidipes* Austen and *G. f. fuscipes* Newstead using combinations of acetone, octenol and cow urine or alone at different dispense rate at Sor Hydrostation.

## II. Materials and Methods

### a) Study area

The study was performed in August-September, 2004 in Baro Akobo river system at Sor River around Sor hydroelectric station at position of 08°.23.06"N-08°.24.03"N, 035°.26.05"E-035°.27.02"E and altitude of 1294-1657m. The area is located at about 630 Km southwest of Addis Ababa, capital of Ethiopia, where *G. pallidipes* and *G .f. fuscipes* are abundant. The valley vegetation consists of very dense riverine and evergreen forests. Wild lives are abundant in the area and includes buffaloes, bushbuck, warthog, colobus monkey, baboon, python, etc.

### b) Study protocol

Five sites, termed as A, B, C, D and E, were located independently for both species in the forest and near Sor River at about 200m distance interval. All sites were provided with new monopyramidal traps of the same type and size which operate throughout the experiment period. Acetone, octenol and cow urine were used as odour attractants at different dispense rate. The natural odour source, cow urine, was obtained from local zebu cattle. After collection, the cow urine was stored at normal room temperature in stopper bottles up to use for 7 days.

Dose rates were measured by recording the decrease in volume of the attractant over the duration of the experiments and converting to milligrams per hour according to FAO (1992). Acetone was dispensed at different dose rates (150-2000mg/hr) by varying the aperture size of the container. The universal glass bottles were drilled in the lid with single holes 0.2 cm, 0.6 cm and 1.8 cm in diameter to release 150mg/hr, 500mg/hr and 2000mg/hr acetone, respectively at 32°C. Octenol was dispensed at 0.5mg/hr from streptomycin glass bottles, containing 2g octenol and sealed with a rubber septum. Octenol containers were positioned horizontally on their sides or inverted always to keep the octenol in contact with the inner surface of the septum and the octenol diffused out through the septum. The cow urine dispensed by the plastic container of 1 litter volume with 4x4 cm cut on its side to give urine release rate of 1000mg/hr. The dispensers were similar and positioned just beneath the trap. The underneath of trap poles were greased to prevent entrance of ants that may eat flies.

### c) Study design

A 5x5 Latin square design (Table 1) replicated three times from 7:00 am to 7:00 am (for 24 hrs) for 15 adjacent days was undertaken each for study of *G. pallidipes* as well as *G. f. fuscipes*. The term 'replicate' denotes the operation of one treatment at one site for one day. Thus, groups of five adjacent days were regarded as different replicates. Blocks and treatments were allotted randomly to days within these blocks.

*Table 1 :* 5x5 Latin square design

| Latin Square | Day | Site | | | | |
|---|---|---|---|---|---|---|
| | | A | B | C | D | E |
| First | 1 | V | II | III | IV | I |
| | 2 | III | I | IV | II | V |
| | 3 | II | IV | I | V | III |
| | 4 | I | V | II | III | IV |
| | 5 | IV | III | V | I | II |
| Second | 1 | II | IV | III | V | I |
| | 2 | I | V | II | III | IV |
| | 3 | IV | I | V | II | III |
| | 4 | III | II | IV | I | V |
| | 5 | V | III | I | IV | II |
| Third | 1 | V | III | II | IV | I |
| | 2 | IV | II | III | I | V |
| | 3 | I | IV | V | III | II |
| | 4 | II | I | IV | V | III |
| | 5 | III | V | I | II | IV |

*Treatment keys: For G. pallidipes: I = trap alone, II = trap + 1000mg/hr cow urine, III = trap + 1000mg/hr cow urine +0.5mg/hr octenol + 150mg/hr acetone, IV = trap + 1000mg/hr cow urine + 0.5mg/hr octenol + 500mg/hr acetone, V = trap + 0.5mg/hr octenol + 2000mg/hr acetone.*
*For: G. f. fuscipes: I = trap alone, II = trap + 0.5mg/hr octenol, III = trap + 500mg/hr acetone, IV = trap + 1000mg/hr cow urine, V = trap + 1000mg/hr cow urine + 0.5mg/hr octenol + 500mg/hr acetone.*

In the experiment for *G. pallidipes,* comparison of unbaited monopyramidal traps versus traps baited with 1000mg/hr cow urine; 1000mg/hr cow urine + 0.5mg/hr octenol + 150mg/hr acetone; 1000mg/hr cow urine + 0.5mg/hr octenol + 500mg/hr acetone; and 0.5mg/hr octenol + 2000mg/hr acetone were used. For *G. f. fuscipes* comparison of unbaited monopyramidal traps versus traps baited with 0.5mg/hr octenol; 500mg/hr acetone; 1000mg/hr cow urine separately, and combinations of 1000mg/hr cow urine + 0.5mg/hr octenol + 500mg/hr acetone were used. To compare the efficacies of various treatments, each treatment was incorporated into a series of randomized Latin square of treatments by sites and by days.

*d) Data analysis*

The catches in each replicate were transformed to log n+1 and subjected to analysis of variance to determine the probability associated with differences between mean catches. The effects of various odours on the catch of trap were compared with a control treatment consisting of unbaited traps. The detransformed mean catch with a test odour is expressed as a proportion of the detransformed mean control (no odour) catch and is termed the catch index; indices greater than unity indicate attraction and indices less than the unity indicate repellent. Presence of statistical significance difference was determined at $p < 0.05$. The critical F ratio is looked up in statistical table.

## III. Results

*a) Glossina pallidipes* Austen

The treatment factor was found as a significant source of variation ($F=29.36$; critical value=2.52; $p<0.05$). Analysis of variance result of *G. pallidipes* at different rates of acetone together with fixed cow urine and octenol doses is shown in Table 2.

*Table 2 :* ANOVA of *G. pallidipes*

| Source of variation | df | SS | MS | F ratio |
|---|---|---|---|---|
| Replicates | 2 | 0.5257 | 0.2629 | |
| Days within replicates | 12 | 3.9709 | 0.3309 | |
| Treatments | 4 | 9.6883 | 2.4221 | 29.3588 |
| Sites | 4 | 1.48 | 0.37 | 4.4848 |
| Residuals | 52 | 4.2885 | 0.0825 | |
| Total | 74 | | | |

The traps baited with different odour attractants have shown better attraction indices than unbaited traps. The treatment with 1000 mg/hr cow urine + 0.5 mg/hr octenol + 500 mg/hr acetone increased the catch highly by up to 12.54 times, and similarly treatment of trap with 1000mg/hr cow urine + 0.5mg/hr octenol +150 mg/hr acetone increased the catch by 11.26 times. Trap baited with highest release rate of acetone (2000mg/hr) + octenol (0.5mg/hr), and 1000mg/hr cow urine alone increased the catch by 4.07 and 2.64 times, respectively (Table 3).

*Table 3 :* The means, detransformed means and indices of each treatment increase in relation to T-I treatment (control) for *G. pallidipes* Austen

| *Glossina pallidipes* treatment type | Mean (x) | Detransformed mean(G) | Index |
|---|---|---|---|
| T-I | 0.5137 | 2.2636 | 1 |
| T-II | 0.8441 | 5.9839 | 2.6435 |
| T-III | 1.4231 | 25.4911 | 11.2613 |
| T-IV | 1.4683 | 28.3968 | 12.545 |
| T-V | 1.0096 | 9.2235 | 4.0747 |

*b) Glossina fuscipes fuscipes* Newstead

The treatment factor was considered as insignificant source of variation ($F=0.6375$; critical value=2.52, $p>0.05$) while site was considered as source of variation ($F=26.6982$; critical value=2.52, $p<0.05$). The results of the experiment comparing trap baited with 0.5 mg/hr octenol, 500 mg/hr acetone, and 1000mg/hr cow urine separately and their combination to catch *G. fuscipes fuscipes* are given in Table 4.

*Table 4 :* ANOVA of *G. f. fuscipes* Newstead

| Source of variation | df | SS | MS | F ratio |
|---|---|---|---|---|
| Replicates | 2 | 0.0627 | 0.0314 | |
| Days within replicates | 12 | 1.4722 | 0.1227 | |
| Treatments | 4 | 0.1426 | 0.0357 | 0.6375 |
| Sites | 4 | 5.9803 | 1.4951 | 26.6982 |
| Residuals | 52 | 2.9137 | 0.056 | |
| Total | 74 | | | |

Since treatment factor was found statistically insignificant, calculating further for index of attraction is not necessary to identify better odour attractant. However, to check the suspicion of their repellent effects was further calculated for indices (Table 5) and found non-repellent roughly.

*Table 5 :* The means, detransformed means and indices of each treatment increase in relation to T-I treatment (control) for *G. f. fuscipes* Newstead

| *G. f. fuscipes* treatment type | Mean (x) | Detransformed mean (G) | Index |
|---|---|---|---|
| T-I | 0.8924 | 6.8055 | 1 |
| T-II | 0.893 | 6.8163 | 1.0016 |
| T-III | 0.9655 | 8.2363 | 1.2102 |
| T-IV | 0.8278 | 5.7267 | 0.8415 |
| T-V | 0.8925 | 6.8073 | 1.0003 |

## IV. Discussion

The efficiency of the traps to catch *G. pallidipes* was high in presence of odour attractants and low in absence of any odour. However, the efficiency was roughly low in the presence of odour for *G. f. fuscipes*. This agrees with the compiled reports of FAO (1992) from many African countries.

*a) Glossina Pallidipes* Austen

Monopyramidal trap was baited with combination of cow urine (1000mg/hr) + octenol (0.5 mg/hr) + acetone (150 mg/hr) could be the preferred combination of odour attractant in the study even though traps baited with cow urine (1000mg/hr) + octenol at 0.5 mg/hr + acetone (500 mg/hr) performed better. This is due to the expensive cost of acetone at high release rate (FAO, 1992). However, in some small operations like ecological researches the later could be utilized.

In the current study, utilization of baited traps with cow urine alone and different combinations of cow urine, octenol and acetone increased the indices (2.64-12.54 times) of the odours' ability to attract *G. pallidipes*. This agrees with work of Dransfield *et al*. (1986) in Ngurman, Kenya, in which acetone with cow urine produced increases of catch 9-25 times using biconical traps. However, the present result disagrees with the result obtained by baiting F3 traps with acetone at 5-50,000 mg/hr release rate or octenol and cow urine, in which indices increased only up to 1.67 in Somalia (Torr *et al*, 1989). The difference may due to variation in the combination of the odour attractants, release rate and geographical location (FAO, 1992).

The result of the current study indicated increment of index up to 4.07 when acetone (2000 mg/hr) and octenol (0.5 mg/hr) were used together. There is a report of slight increase in the effects of octenol and acetone even though it was statistically not significant in Kenya (Baylis and Nambiro, 1993). However, acetone and octenol dispensed either alone or together did not significantly increase the catch of a trap in Somalia (Vale and Hall, 1985b). According to FAO (1992), using acetone with octenol can double the catch when compared with octenol used alone.

Traps baited with cow urine (1000mg/hr) alone increased catch index up to 2.64 times when compared with unbaited traps in this study. This is slightly in line of agreement with work of Vale and Hall (1985b) in which a mixture of 3-n-propylphenol and 4-methylphenol increased the catch index by 1.5-2 times. According to FAO (1992), 4-methylphenol and 3-n-propylphenol are the most active components in the urine and bovid urine can give substantial increases in catch (2-5) if dispensed at about 1000mg/hr. If a much higher rate of urine is used, it becomes repellent.

Traps baited with 1000mg/hr cow urine + 0.5mg/hr octenol + 150mg/hr acetone and 1000mg/hr cow urine + 0.5mg/hr octenol + 500mg/hr acetone increased catch index to 11.26 and 12.54 times, respectively. The current catch index result is greater than results (catch index 4-6 baited traps over unbaited traps) obtained from a combination of acetone (500mg/hr), octenol (0.8 or 1.5mg/hr), mixture of 4-methylphenol and 3-n-propylphenol (0.8mg/hr) at Galana Ranch, south-eastern Kenya (Baylis and Nambiro, 1993). In addition, the combination of the odours of current study showed much greater catch index than combination of 3-methylphenol, acetone and octenol, which increased the catch 1.6 times in Zimbabwe using F3 traps (Hall *et al*., 1990). However, the current result is less than a combination of acetone,

octenol, 4-methylphenol and 3-prophylphenol that increased catching up to 20 times in Zimbabwe (Vale and Hall, 1985a; Vale *et al.*, 1988). Performance difference of different traps to attract tsetse flies were found minimal ((Baylis and Nambiro, 1993) and this rules out effects of different traps. Therefore, the difference might be due to difference in geographical location and release rate.

*b) Glossina fuscipes fuscipes* Newstead

Various ketones, octenol, urine and phenols have been tested but results have been inconsistent (FAO, 1992). There was no statistically significant difference in attractiveness of cow urine to *G. f. fuscipes* in the current study. However, there was a significant increase catch of blue-black polyethylene bipyramid traps using local zebu urine (x 1.4) in three of the four trails with the greatest effect (x 4.2) obtained for male *G. f. fuscipes* when the densities of flies were low (less than five male per trap per day) in Central African Republic. The difference between these works may be related to particular environment of the trails (Gouteux *et al.*, 1995).

Odour attractants used alone or in combination in the current study has not significantly increased the catch of *G. f. fuscipes*. According to FAO (1992), the search for odour attractants for this species has so far been unsuccessful, with the exception of carbon dioxide. This is due to the fact that the riverine tsetse species of palpalis-group respond poorly to the odour baits developed for savannah species. The attractants used for the savannah tsetse are hardly or not attractive to palpalis species and sometimes even repel them (Mwangelwa *et al.*, 1995; Späth, 1995). So a full understanding of the types of odour attractants and interplay of responses of *G. f. fuscipes* to different chemicals and suggestion of effective odour bait has to be worked out in the future.

## V. Conclusion and Recommendations

In conclusion, different combinations of odour attractants were found to attract *G. pallidipes*. To run some small operations such as ecological research for *G. pallidipes*, traps are required to provide large samples with small cost and convenience. In these circumstances, an appropriate odour would be combination of medium doses of 500mg/hr acetone + 0.5 mg/hr octenol + 1000mg/hr cow urine. For works such as routine surveys or control operations over large areas, a less expensive and more convenient combination of low dose of 150 mg/hr acetone + 0.5 mg/hr octenol and 1000mg/hr cow urine would be more suitable. The latter combination can increase catches by several times for costs that are much less than the equally effective alternative of operating higher doses of odour or more traps. The search for best odour attractant for *G. f. fuscipes* is unsuccessful in the current study. Thus, further studies should be conducted on the behavior and best odour attractant for this species.

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## VII. Contribution of the Authors

WT conceived the idea, participated in all field works, analysis and write up of the manuscript. AO participated in all field works and FT participated in data analysis and write up of the manuscript.

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# Effect of High Energy and High Protein Diets on Zinc and Copper Metabolism in Goats

By A.N.Sayed, Nabila A.Gazia, A.M.Abd-Ellah & Sh. M.Abdel-Raheem

*Abstract-* The effect of different levels of energy and protein on the utilization of zinc and copper were evaluated in four metabolic experiments. A total of nine growing castrated male balady kids having nearly the same age (8-9 months) and body weight (15-18kg) were experimented on. Kids were housed individually in metabolism cages in order to collect feces and urine. Four experiments, 3 trials per each were done on the same animals, with about 10 days as a rest period between one experiment and another, in which the animals were fed on control ration during the interval. The nine kids were randomly divided into 3 groups (A, B and C), 3 kids per each. The first group (A) was fed the control ration and used as control, while the other two groups (B and C) were fed the tested rations which furnished 15% more or less DE and CP than the control. Feeding the high energy ration increased the apparent absorption and retention of copper and decreased the apparent retention of zinc, while feeding low energy ration decreased the apparent absorption and retention of zinc and copper.

*Keywords:* *energy, protein, zinc, copper, metabolism, goats.*

*GJMR-G Classification :* *NLMC Code: QU 55, WB 400*


*Strictly as per the compliance and regulations of:*

# Effect of High Energy and High Protein Diets on Zinc and Copper Metabolism in Goats

A.N.Sayed [α], Nabila A.Gazia [σ], A.M.Abd-Ellah [ρ] & Sh. M.Abdel-Raheem [ω]

*Abstract-* The effect of different levels of energy and protein on the utilization of zinc and copper were evaluated in four metabolic experiments. A total of nine growing castrated male balady kids having nearly the same age (8-9 months) and body weight (15-18kg) were experimented on. Kids were housed individually in metabolism cages in order to collect feces and urine. Four experiments, 3 trials per each were done on the same animals, with about 10 days as a rest period between one experiment and another, in which the animals were fed on control ration during the interval. The nine kids were randomly divided into 3 groups (A, B and C), 3 kids per each. The first group (A) was fed the control ration and used as control, while the other two groups (B and C) were fed the tested rations which furnished 15% more or less DE and CP than the control. Feeding the high energy ration increased the apparent absorption and retention of copper and decreased the apparent retention of zinc, while feeding low energy ration decreased the apparent absorption and retention of zinc and copper. Feeding the high protein ration decreased the apparent absorption and retention of copper, while low protein ration increased the absorption and retention of zinc and copper. Feeding the high energy-high protein ration increased the apparent absorption of zinc but decreased the apparent absorption of copper. On the other hand, low energy-low protein ration decreased the absorption and retention of zinc but increased the absorption and retention of copper.

*Keywords: energy, protein, zinc, copper, metabolism, goats.*

## I. Introduction

There is no doubt that considerable increase in animal production can be achieved with improved nutrition and management practices under different production systems of management [1]. Efficient utilization of nutrients depends on an adequate supply of energy, which of paramount importance in determining the productivity. In goats, energy deficiency retards kids growth, delays puberty, reduces fertility[2]. With continued energy deficiency, the animals show a concurrent reduction in resistance to infectious diseases and parasites. The problem may be further complicated by deficiencies of protein, minerals and vitamins. Energy limitation may result from inadequate feed intake. Low energy intake that result from either feed restriction or low diet component digestibility prevent goats from meeting their requirements and from attaining their genetic potential. Goats are more active and travel greater distances than sheep, which increases energy requirements, high water content of forages may also become a limiting factor [3]. Protein deficiencies in the diet deplete stores in the blood, liver and muscles and predispose animal to a variety of serious and even fatal aliments. The further protein deficiency reduces rumen function and lower the efficiency of feed utilization [2]. Mineral requirements for animals is affected by many aspects, such as nature and level of production, age, level and chemical form of elements, breed and animal adaptation[4]. The bioavailability of trace-elements to animals can be affected by a variety of dietary components, one of these components is protein[5]. Copper deficiency is a serious problem for grazing ruminant in many countries of the world due to both low concentration of the element in the forage as well as to elevated amount of molybdenum and sulfur which interfere with copper utilization[6]. The present study was carried out to investigate the effect of different levels of energy and protein on the metabolism of zinc and copper.

## II. Materials and Methods

### a) Animals and housing

A total number of nine growing castrated male balady kids to be nearly of the same age (8-9 months) and body weight (15-18 kg) were used in this study. Each kid was kept in an individual metabolic cage allowing the collection of feces and urine separately. A weighed daily ration was offered to each animal in its respective feed trough and tap water freely available.

### b) Rations and Feeding

This study was carried out in four experiments, each experiment had 3 trials and each trial durated 30 days. Before starting the experiments, the kids were fed a balanced ration (control ration) for three weeks in order to accustom the animals on the ration and to assume the repletion of body mineral store. The control ration was formulated to contain the recommended levels of digestible energy (DE) 2.94 Mcal/kg, crude protein (CP) 9.51%, zinc 63.55 ppm and copper 16.72 ppm according to the NRC3 for goats. The preliminary period was extended for 21 days, while the collection period was extend for 8 days. During the collection period, the daily fecal matter excreted and the daily amount of urine were separately collected, measured, sampled and prepared for further chemical analysis. A

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fixed weight of the ration was offered to each animal and the daily feed intake was calculated. The experiments were carried on the same groups and separated by a rest period of 10 days, during which the kids were fed on control ration. Nine test rations varying in their energy and protein levels were fed to the kids during the metabolism study as shown in the design in Table 1. The nine kids were randomly divided into 3 groups (A, B and C), 3 kids per each. The first group (A) was fed the control ration and used as control, while the other two groups (B and C) were fed the tested rations which furnished 15% more or less DE and CP than the control. The physical and chemical composition of the nine tested rations and energy values (DE) were shown in Tables 3 and 4, while Table 2 shows the chemical composition and energy value of the ingredients used in formulating of the experimental rations.

*Table 1 :* The design of experimental study

| Experiment | Trial | Group | DE (Mcal/kg diet) | CP (%) | Ration |
|---|---|---|---|---|---|
| I | 1 | A | 2.94 | 9.51 | 1 NENP* |
| | 2 | B | 2.96 | 10.79 | 2 NEHP |
| | 3 | C | 2.93 | 8.08 | 3 NELP |
| II | 1 | A | 2.94 | 9.51 | 1 NENP |
| | 2 | B | 3.39 | 9.54 | 4 HENP |
| | 3 | C | 3.40 | 11.00 | 5 HEHP |
| III | 1 | A | 2.94 | 9.51 | 1 NENP |
| | 2 | B | 3.37 | 8.14 | 6 HELP |
| | 3 | C | 2.53 | 9.59 | 7 LENP |
| IV | 1 | A | 2.94 | 9.51 | 1 NENP |
| | 2 | B | 2.58 | 10.80 | 8 LEHP |
| | 3 | C | 2.51 | 8.06 | 9 LELP |

**NE=normal energy, HE=High energy, LE=Low energy, NP=normal protein, HP=high protein, LP=low protein*

*Table 2 :* Chemical composition (on DM basis) and digestible energy of the ingredients used in the experimental rations

| Ingredient | DM | Chemical composition | | | | | | | | DE (Mcal/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (%) | | | | | | (ppm) | | |
| | | OM | CP | EE | CF | NFE | Ash | Cu | Zinc | |
| Yellow corn | 89.7 | 97.88 | 9.6 | 4.16 | 2.77 | 81.35 | 2.12 | 3.5 | 12.8 | 3.84 |
| Soybean meal | 91.3 | 93.09 | 47.0 | 5.47 | 7.44 | 33.18 | 6.91 | 22.8 | 42.9 | 3.88 |
| CSM | 92.5 | 95.40 | 27.0 | 6.40 | 24.5 | 37.50 | 4.60 | 19.9 | 62.2 | 2.65 |
| Wheat bran | 90.65 | 93.00 | 15.6 | 4.70 | 8.37 | 64.33 | 7.00 | 12.7 | 113.7 | 3.09 |
| Wheat straw | 90.0 | 86.70 | 3.5 | 1.66 | 38.0 | 43.54 | 13.30 | 3.2 | 5.6 | 1.94 |
| Limestone | 98.0 | - | - | - | - | - | 100 | - | - | - |
| Common salt | 98.0 | - | - | - | - | - | 98.00 | - | - | - |
| Mineral mixture | 98.0 | - | - | - | - | - | 98.00 | 10 | 40 | - |
| AD3E | 98.0 | - | - | - | - | - | - | - | - | - |

*Table 3 :* Physical composition of the experimental rations

| Ingredients | Rations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | NENP | NEHP | NELP | HENP | HEHP | HELP | LENP | LEHP | LELP |
| Yellow corn | 46.00 | 43.00 | 50.00 | 74.00 | 70.00 | 77.00 | 18.00 | 20.00 | 23.00 |
| Soybean meal | 5.00 | 10.00 | 2.00 | 2.00 | 7.00 | - | 8.00 | 10.00 | 3.00 |
| Cottonseed meal | 2.00 | - | 2.00 | 2.00 | - | - | 2.00 | 5.00 | 5.00 |
| Wheat bran | 5.00 | 3.00 | 2.00 | 2.00 | 2.00 | - | 9.00 | 5.00 | 6.00 |
| Wheat straw | 40.45 | 42.55 | 42.45 | 18.35 | 1.35 | 21.35 | 61.65 | 58.65 | 61.55 |
| Limestone, ground | 0.80 | 0.70 | 0.80 | 0.90 | 0.90 | 0.90 | 0.60 | 0.60 | 0.70 |
| Common salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| AD3E | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral mixture | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

*Table 4 :* Chemical composition on (DM basis) and digestible energy of the experimental rations

| Rations | DM | Chemical composition | | | | | | | | DE (Mcal/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ( % ) | | | | | | ( ppm ) | | |
| | | OM | CP | EE | CF | NFE | Ash | Cu | Zinc | |
| 1 | 90.14 | 91.41 | 9.51 | 3.22 | 17.92 | 60.76 | 8.59 | 16.72 | 63.55 | 2.94 |
| 2 | 90.14 | 91.19 | 10.79 | 3.19 | 18.36 | 58.85 | 8.81 | 17.23 | 61.72 | 2.96 |
| 3 | 90.07 | 91.48 | 8.08 | 3.11 | 18.33 | 61.96 | 8.52 | 15.79 | 59.07 | 2.93 |
| 4 | 90.01 | 94.08 | 9.53 | 3.71 | 9.83 | 71.01 | 5.92 | 15.88 | 60.98 | 3.39 |
| 5 | 90.03 | 93.79 | 11.00 | 3.70 | 9.98 | 69.11 | 6.21 | 16.57 | 61.47 | 3.40 |
| 6 | 89.91 | 93.99 | 8.14 | 3.55 | 10.24 | 72.06 | 6.01 | 14.88 | 56.80 | 3.37 |
| 7 | 90.27 | 88.91 | 9.59 | 2.76 | 25.77 | 50.79 | 11.09 | 17.68 | 67.26 | 2.53 |
| 8 | 90.34 | 89.27 | 10.80 | 2.91 | 25.23 | 50.33 | 10.73 | 18.25 | 65.30 | 2.58 |
| 9 | 90.25 | 89.11 | 8.06 | 2.74 | 25.98 | 52.33 | 10.89 | 16.86 | 63.97 | 2.51 |

*c) Fecal samples*

The total amount of the daily fecal matter excreted per animal was collected daily at 8.00am before feeding. The freshly collected fecal matter of each animal was weighed, recorded, mixed thoroughly and then representative sample (10%) was taken and dried in hot air oven at 60C for about 24h. The dried fecal samples from each animal were thoroughly mixed finely ground and stored at room temperature for further chemical analysis.

*d) Urine samples*

The daily urine excreted by the kids was collected at 8.00am and measured in graduated cylinder to record its volume. The collected amount from each animal was then thoroughly mixed and two representative samples, 100ml each was taken and acidified with 2ml of concentrated hydrochloric acid, then kept in refrigerator at 4°C for further chemical analysis.

*e) Metabolism trials*

In the simple form of the balance technique, the intake of the element under study is compared with the fecal output from the animal's body and the difference is assumed to be absorbed by the animal (apparent absorption) which may then be expressed as a percentage of the dietary intake or in g or mg/head/day.

$$\text{Apparent absorption} = \frac{\text{Intake - fecal excretion}}{\text{Intake}} \times 100$$

$$\text{Apparent retention} = \frac{\text{Intake} - (\text{fecal excretion} + \text{urinary excretion})}{\text{Intake}} \times 100$$

(Ammerman et al)[6]

*f) Minerals determination in feces and urine*

Duplicate samples of 1gm feces and 10ml of urine were ashed with 20ml acid mixture (2 parts concentrated nitric acid + 1 part concentrated perchloric acid) and then digested on hot plate for 1.5-2h until the color become clear and volume reduced to the minimum. The ashed samples were diluted with bidistilled water in clean dry tightly closed glass bottles 100ml capacity and then stored for subsequent minerals determination. The zinc and copper in the prepared samples of fecal matter were measured in ppm and of urine in mg/L by atomic absorption/flame emission spectrophotometer using an air-acetylene flame and hallow cathode lamp after method described by Slavin [8].

## III. Results and Discussion

The metabolic balances of zinc and copper in the four experiments are presented in Tables 5 and 6.

*Table 5 :* Zinc balance (mg/head/day) in kids during the experiments

| Item | Experiment I | | | Experiment II | | | Experiment III | | | Experiment IV | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NENP | NEHP | NELP | NENP | HENP | HEHP | NENP | HELP | LENP | NENP | LEHP | LELP |
| Zn intake | 29.26 | 30.08 | 29.55 | 31.84 | 30.47 | 32.38 | 32.7 | 30.61 | 35.63 | 34.42 | 35.53 | 35.61 |
| Fecal Zn | 17.43 | 17.7 | 17.41 | 18.87 | 17.95 | 19.00 | 19.31 | 17.77 | 21.09 | 20.02 | 20.74 | 20.80 |
| Urinary Zn | 3.00 | 3.05 | 2.96 | 2.94 | 2.97 | 3.18 | 2.84 | 2.76 | 3.03 | 2.90 | 2.98 | 2.94 |
| Absorbed Zn | 11.83 | 12.37 | 12.14 | 12.97 | 12.52 | 13.38 | 13.57 | 13.02 | 14.54 | 14.40 | 14.79 | 14.81 |
| Retained Zn | 8.83 | 9.32 | 9.18 | 10.03 | 9.55 | 10.20 | 10.73 | 10.26 | 11.51 | 11.5 | 11.81 | 11.87 |
| Absorption | 30.43 (100)* | 41.12 (101.17) | 41.08 (101.61) | 40.73 (100) | 41.08 (100.86) | 41.32 (101.45) | 41.5 (100) | 42.52 (102.46) | 40.81 (98.34) | 41.84 (100) | 41.63 (99.50) | 41.59 (99.4) |
| | 30.18 | 30.98 | 31.07 | 31.50 | 31.34 | 31.50 | 32.81 | 33.52 | 32.30 | 33.41 | 33.24 | 33.33 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % Retention % | (100)* | (102.65) | (102.95) | (100) | (99.49) | (100) | (100) | (102.16) | (98.45) | (100) | (99.49) | (99.76) |

*Absorbed = intake – fecal Retained = intake – (fecal + urinary)*

**Figures in parentheses are the rate of absorption or retention in percentages.*

*Table 6 :* Copper balance (mg/head/day) in kids during the experiments

| Item | Experiment I | | | Experiment II | | | Experiment III | | | Experiment IV | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NENP | NEHP | NELP | NENP | HENP | HEHP | NENP | HELP | LENP | NENP | LEHP | LELP |
| Cu intake | 8.54 | 9.32 | 8.77 | 9.29 | 8.81 | 9.70 | 9.54 | 8.92 | 10.37 | 10.05 | 10.99 | 10.40 |
| Fecal Cu | 4.24 | 4.74 | 4.28 | 4.66 | 4.36 | 4.90 | 4.73 | 4.40 | 5.18 | 4.99 | 5.70 | 5.16 |
| Urinary Cu | 0.65 | 0.79 | 0.68 | 0.88 | 0.79 | 0.86 | 0.82 | 0.83 | 0.87 | 0.95 | 1.09 | 0.93 |
| Absorbed Cu | 4.30 | 4.58 | 4.49 | 4.63 | 4.45 | 4.80 | 4.81 | 4.52 | 5.19 | 5.06 | 5.29 | 5.24 |
| Retained Cu | 3.65 | 3.79 | 3.81 | 3.75 | 3.66 | 3.94 | 3.99 | 3.69 | 4.30 | 4.11 | 4.20 | 4.31 |
| Absorption % | 50.35 | 49.14 | 51.20 | 49.84 | 50.51 | 49.48 | 50.42 | 50.67 | 50.05 | 50.35 | 48.13 | 50.38 |
| Retention % | (100)* | (97.6) | (101.69) | (100) | (101.34) | (99.28) | (100) | (100.50) | (99.27) | (100) | (95.59) | (100.06) |
| | 42.74 | 40.66 | 43.44 | 40.36 | 41.54 | 40.62 | 41.82 | 41.37 | 41.47 | 40.8 | 38.22 | 41.44 |
| | (100)* | (95.13) | 101.64) | (100) | (102.92) | (100.64) | (100) | (98.92) | (99.16) | (100) | (93.68) | (101.57) |

*Absorbed = intake – fecal Retained = intake – (fecal + urinary)*

**Figures in parentheses are the rate of absorption or retention in percentages.*

*Experiment I*

Kids group fed high protein showed slight increase in the amount of zinc intake and in the excreted zinc in both feces and urine compared with control. The increased urinary Zn excretion by high protein diet was similar to the findings of Greger and Snedeker[5] who reported that high protein diet increased urinary zinc excretion and attributed that to a greater amount of histidine and cystine in the high protein ration. The apparent absorption and retention of Zn were increased when dietary protein levels increased as reported by Gawthorne et al[9]. Feeding high or low protein rations increased the amount of Cu intake, Cu excretion in feces and urine compared to control. The apparent Cu absorption and retention percentages were decreased in kids group fed the NEHP ration, while increased in group fed on NELP ration compared with control. The increase dietary crude protein is responsible for the formation of insoluble copper sulfide during rumen fermentation resulting in lower solubility and absorption of Cu [10,11].

*Experiment II*

High energy-high protein ration slightly increased the amount of Zn intake, fecal and urinary Zn excretion than control one. The increase of fecal Zn excretion in kids fed HEHP ration may be due to high Zn intake as reported by McDowell12 who reported that the fecal endogenous Zn increases with the increased Zn intake. The apparent Zn absorption was increased in HENP & HEHP rations, while the apparent retention was decreased in kids group fed HENP. Feeding high energy ration decreased the amount of Cu intake, fecal and urinary Cu excretion compared to control. The apparent absorption and retention of Cu were slightly increased in kids group fed HENP ration.

*Experiment III*

Feeding the HELP ration was decreased the amount of Zn intake, zinc excreted in feces and urine compared to control one. The apparent Zn absorption and retention were increased in group fed HELP ration, while decreased in group fed LENP. The decrease in absorption percentage in low energy ration may be due to high zinc intake [13]. Feeding low energy ration increased the amount of Cu intake, fecal and urinary excretion compared to HELP and control one. The apparent absorption and retention of Cu were decreased in kids group fed LENP ration.

*Experiment IV*

Kids fed the LEHP and LELP rations showed a slight increased in the amounts of Zn intake, Zn excreted in both feces and urine compared to control. The apparent absorption and retention of Zn were decreased in kids group fed the LEHP and LELP rations compared to control. On this respect, many authors reported that, the apparent Zn absorption and retention were increased when dietary protein levels were increased 9. Kids fed on LEHP ration recorded the highest amount of Cu intake and excretion in feces and urine compared to LELP and control one. The apparent absorption and retention were decreased in kids group fed the LEHP compared to the control one. These findings were in accordance with that found by Ward 10 and Ivans and Veira11 who found that the increase in dietary CP resulting in lower solubility and absorption of Cu in sheep. The summarized effect of energy and protein levels revealed that feeding HENP ration increased the apparent absorption and retention of Cu, while the NEHP and LEHP rations recorded the lowest values for apparent absorption and retention compared to other groups including control. On the other hand, feeding low

protein with high energy levels increased the apparent absorption of Zn compared to the control group.

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# Effects of *Trypanosoma Brucei* on Some Electrocardiographic Repolarisation Indices of Dogs

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Ten dogs inoculated with 1 ml of Phosphate buffered saline diluted blood containing 1x106 of the federe strain of the parasite had their ECG recorded on weekly basis for three weeks. Plasma potassium concentration was assayed in each dog before the electrocardiogram.

Supraventricular arrhythmia, ventricular premature contraction, ventricular tachycardia and various degrees of Atrioventricular blocks were recorded from the eighth day of infection. T. brucei infected dogs had elevated heart rate during the period of infection in all the six leads studied. At various times during the infection, QT and QTC of the infected dogs were significantly lower than the uninfected ones in leads II and AVF.

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*GJMR-G Classification : NLMC Code: WG 140, FOR Code: 070799*


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# Effects of *Trypanosoma Brucei* on Some Electrocardiographic Repolarisation Indices of Dogs

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*Summary-* The effect of acute *T.brucei* infection on some ECG repolarisation indices like QT dispersion ($QT_D$), heart rate corrected QT dispersion ($QT_{CD}$), T wave voltage expressed as T/R, Mean electrical axis (MEA), Heart rate (HR) and plasma potassium concentration was evaluated in dogs.

Ten dogs inoculated with 1 ml of Phosphate buffered saline diluted blood containing $1x10^6$ of the *federe* strain of the parasite had their ECG recorded on weekly basis for three weeks. Plasma potassium concentration was assayed in each dog before the electrocardiogram.

Supraventricular arrhythmia, ventricular premature contraction, ventricular tachycardia and various degrees of Atrioventricular blocks were recorded from the eighth day of infection. *T. brucei* infected dogs had elevated heart rate during the period of infection in all the six leads studied. At various times during the infection, QT and $QT_C$ of the infected dogs were significantly lower than the uninfected ones in leads II and AVF. Although the T wave voltage was also increased in the infected dogs, the $QT_D$ $QT_{CD}$, and the MEA were not affected by the infection. The $QT_D$ and the $QT_{CD}$ indices of arrhythmic dogs were however found to be significantly lower than in the non-arrhythmic dogs. The serum potassium concentration of the infected dogs was significantly lowered in the first two weeks of infection and then rose to the control level during the third week of infection. Serum potassium concentration in arrhythmic dogs was however not different from the non-arrhythmic ones. The increased heart rate and the shortened QT and $QT_C$ width seen during the course of the infection reflect the enhanced state of sympathetic activity and the propensity for arrhythmia in the infected dog.

This result has shown that ECG changes and arrhythmia seen in this study may not strictly reflect structural and functional cardiac involvement but changes in ANS functions coupled with perturbation in electrolyte and the metabolic statues of the infected dogs may have also been implicated. The usefulness of the $QT_D$, $QT_{CD}$, as a sole marker for the detection of cardiac abnormality is also limited because of the inherent technical problems associated with the measurement of QT.

*Keywords: ECG, qtdispersion, arrhythmia, t. brucei, canine.*

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## I. Introduction

Canine trypanosomiasis like canine babesiosis, chagas disease and malaria is characterized by myocarditis which often causes arrhythmia ( Dvir *et al.,* 2004; Sprague, 1946; Zhang *et al.,* 1999). Arrhythmia and conduction block is caused by a decrease in resting membrane potential of the ischemic myocardial cells (Boyden, 1996).

*T. brucei* infection has been reported to cause gross and microscopic heart lesion starting from the eighth day of infection (Morrison *et al.,* 1983).

Impulse initiation disorders like Ventricular tachycardia and ventricular premature contraction and impulse conduction disturbances like Atrioventricular ventricular block and bundle branch block often characterized *T. brucei* infection in dogs (Ndungu *et al.,* 1991).

Arrhythmias, especially of Ventricular origin normally affects repolarisation indices like QT dispersion ($QT_D$), that is, the range of QT interval duration in all measurable ECG leads and corrected QT dispersion ($QT_{CD}$) which is the difference between the maximum and minimum heart rate corrected QT intervals.

The usefulness of these indices in canine cardiology and canine trypanosomiasis in particular has not been fully exploited. Previous ECG work done on experimental *T. brucei* infection in dogs did not explore the effects of this infection on changes in repolarisation indices, heart axis and plasma electrolyte profile (Ndungu *et al.,* 1991).

In this study, electrocardiogram of *T. brucei* infected dogs will be monitored for changes in cardiac rate rhythms and repolarization indices. And the role of cardiac repolarisation indices as markers of cardiac mortality in canine trypanosomiasis highlighted. This work will apart from addressing the existing knowledge gap between African canine trypanosomiasis and chagas disease, its South American counterpart, it will also provide alternative cardiac indices needed to diagnose and monitor this disease and other related cardiac conditions.

## II. Animals, Materials and Methods

Ten dogs sourced locally from a local breeder in Abeokuta were used for the study. The dogs were

between 3 to 6months old and they weighed between 4−7kg. They were acclimatized for two weeks and screened against trypanosome species and other hemoparasites. All animals that participated in this study showed no evidence of Dirofilariasis or any other cardiac conditions and had received all routine vaccination. Dogs were kept in fly proof kennel, fed twice on commercial dog food (Jojo dog foods, Ikeja) and allowed asses to water *ad libitum.*

This work was conducted in accordance to provisions of the ethical committee of College of Veterinary Medicine, Federal University of Agriculture, Abeokuta.

*T. brucei* (*federe* strain) got from Nigerian Veterinary Reseach Institute, Vom, was used for this study. The parasites were preserved by sub-pass aging in donor albino rats. And each dog serving as its own control was inoculated with 1 ml of phosphate buffered saline diluted blood containing $1\times 10^6$ of the parasites intraperitoneally.

A six-lead (I, II, III, AVR, AVL, AVF) body surface electrocardiogram of dogs placed on standard position was recorded serially before and on days 8, 16, and 24 post infections. The animals were not clipped and contact between skin and electrodes was improved by application of electrode gel. All recordings were made on one of the channels of a four channel universal student Oscillograph (Harvard apparatus, UK) by the same ECG technician. The paper speed was 25mm/sec and the pen sensitivity 10mm=1mV.

The QT interval and the preceding RR interval were measured and averaged in five consecutive P-QRS-T complexes in each lead. These intervals were measured manually to the nearest 0.5mm using calipers and ruler.

The QT interval measured from the beginning of Q to the end of T is defined as the return of T to the isoelectric line (Brooksby *et al.,* 1999). The corrected QT (QTc) was derived with the Fridericia formula; $QTc= QT/RR^{1/3}$(Fridericia, 1920). QT dispersion ($QT_D$) and the heart rate corrected QT dispersion ($QT_{CD}$) were calculated as the difference between the minimum and maximum value of QT and QTc (Dennis *et al.,* 2002). Mean electrical axis of the heart was determined using the lead graphing method (Edwards, 1993). T wave amplitude was evaluated as T/R ratio (Dvir *et al*., 2004). The heart rate was determined by counting the number of cycles (RR interval) in six seconds and multiplying by ten.

Lead II ECG traces obtained from infected and control dogs were analyzed for arrhythmia. The ECG tracings were evaluated for arrhythmia by a panel of cardiologist who were not part of the study.

All data were expressed as Mean ± Standard deviation. Differences within parameters during the course of the disease were evaluated by ANOVA for repeated measures. Statistical significance between the pre- infection control and a value at a particular time point after the infection was determined by paired t- test with bonferoni correction.

The arrhythmia group was compared with the non-arrhythmia group using the t-test for independent sample. $P < 0.05$ was considered significant. All statistical tests were done using SPSS version 16.

## III. Results

A total of 240 electrocardiograms were obtained from ten dogs. The QT and the QTc parameters were measurable in all six leads in one hundred and thirty six of the two hundred and forty electrocardiograms.

A total of 36 out of 40 lead II electrocardiograms were analyzed for arrhythmia. Four ECG were discarded due to its artefactual content. Twenty electrocardiograms representing 55.5% showed various forms of arrhythmias starting from the 8th day of infection. Each of the affected electrocardiograms showed at least one form of arrhythmia. Ventricular premature contractions (VPC), ventricular tachycardia (VT), polymorphic ventricular tachycardia (PVT), bundle branch block (LBBB), Atrioventricular blocks (AVB), notched R wave, ST wave slurring (STS), ST wave depression (STD) and sinoventricular rhythm were shown by the dogs at different times during the study( Figure 1-5).

As shown in table 1, the heart rate, T/R voltage and the plasma potassium were significantly affected by the infection but the mean $QT_D$, $QT_{CD}$, and the MEA, were not affected by the disease progression. Although the mean serum potassium concentration of the infected dogs on the 8th and 16th day of infection was significantly lower than in uninfected dogs, the plasma potassium concentration on the 24th day of infection was significantly higher than on the 8th day ($P\leq 0.01$) and on the 16th day ($P\leq 0.01$).

When the indices in table 1 were compared between arrhythmic and non-arrhythmic dogs, it was revealed in table 2, that T/R voltage was taller in arrhythmic dogs but the $QT_{CD}$ and $QT_D$ were wider in dogs without arrhythmia.

As shown in table 3, *T. brucei* infection caused a reduction in QT and QTc indices. At days 8, 16, and 24 post-infection, QT was significantly reduced compared to control value. The QTc was however significantly shortened only on days 8 and 24 post-infection.

## IV. Discussion

The exhibition of various forms of arrhythmia like Ventricular premature contraction, Ventricular tachycardia, Atrioventricular conduction blocks and Supraventricular arrhythmia in the *T. brucei* infected dogs is consistent with other infections like babesiosis, malaria, and chagas disease (Dvir *et al.,* 2004; Sprague, 1946; Barr *et al.,* 1992). Myocarditis, a common feature of canine trypanosomiasis has been reported by some

workers to provide a suitable substrate for arrhythmia (Boyden, 1996). These arrhythmias are often triggered by enhanced heterogeneity of ventricular repolarisation (Merx *et al.,* 1977; Ahnve and Vallin 1982; Kuo *et al.,* 1983).

Although several workers have reported the association of QT interval prolongation with lethal form of ventricular arrhythmia (ref), the reduced QT and QTc width seen in this study have also been reported to be potentially pro-arrhythmic.

On the surface electrocardiogram, heterogeneity of ventricular repolarisation often manifest as increased $QT_D$, and $QT_{CD}$ (Higham *et al.,* 1992; Day *et al.,* 1990; De-Bruyne *et al.,* 1998). Although the $QT_D$ and $QT_{CD}$ were not affected by disease progression, non arrhythmic dogs have a more dispersed $QT_D$ and $QT_{CD.}$ This is at variance with Dennis *et al.,* 2002, who reported that $QT_{CD}$ index of arrhythmic animals was insignificantly higher than those without arrhythmia. The extent and location of myocardial damage are related to the generation of ventricular arrhythmia (Lown *et al.,* 1969; Kuo *et al*., 1985; Kutz *et al.,* 1994).

Since histopathology was not part of this work, the extent of myocardial damage could not be ascertained. The markedly elevated plasma potassium level on the 24$^{th}$ day of infection however is an indication of possible myocardial damage (Janse and Witt 1989).

This study similarly to the findings of Barbabosa-Pliego *et al* (2009), in *T. cruzi* infection of dogs reported a significantly elevated T/R at a later stage of the infection. When compared with the reference range, the value of T/R either before or after infection was higher than the reference value of T/R≤0.25 (Dvir *et al.,* 2004). The increased amplitude of the T wave in this study may be due to hyperkalemia which was noticed at the later part of the infection (El-Sheriff and Turitto 2011). High amplitude T-waves have been linked to hyperkalemia of myocardial infarction (Feldman and Ettinger 1977).

In agreement with Ndungu *et al* (1991), *T. brucei* infected dogs in this study showed tachycardia. The reduced QT and QTc width exhibited by the infected dogs could result from shortened action potential duration and this could consequently increase the heart rate of the infected dogs. Some workers have reported the role of increase cardiac sympathetic activity in myocardial infarction (Esler and Kaye 2000). In *T. cruzi* infection, rarefaction of the cardiac parasympathetic nerves has been reported (Olivieria, 1985). The increased cardiac sympathetic discharge often seen in myocardial infarction (Jardine *et al.,* 2005) could be the reason for the tachycardia observed in this study.

The Atrioventricular and intramyocardial blocks observed in this study has been previously reported in *T. brucei* and *T. cruzi* infections of dogs (Ndungu *et al.,* 1991; Anselmi *et al.,* 1967).

*T. brucei* infection as seen in this study does not affect the heart's chamber size and axis. Although the MEA of dogs used in this study did not fall within the reference range of 40°–100° as reported by some authors for the specie(Tilley and Larry, 2001; Martin, 2005), they tend to fall within those of humans which have been reported to be between -30°– 90° (Fouchet and Gateff 1968). This probably reflected the breed peculiarity of the Nigerian dogs. The arrhythmia observed in some dogs may therefore not necessarily reflect a primary structural myocardial damage but may be a result of metabolic disturbances which often characterize canine trypanosomiasis. Our observation is thus in agreement with Fouchet and Gateff (1968) who earlier reported that axis deviation is not a common finding in African human Trypanosomiasis.

Although the QT interval was read manually in the present study, the repeatability index of the values obtained was high and the values of the $QT_D$ and $QT_{CD}$ reported here agrees with those that have been reported previously for normal dogs and those with cardiac conditions (Dennis *et al.,* 2002).

For now, because of the inherent technical problems and the inconsistency associated with measurement of QT index, restraint should be exercised in its use as a marker of cardiac mortality in canine trypanosomiasis.

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*Table 1 :* Post Infection Variation In Serum Potassium And Ecg Repolarization Indices Of *T.Brucei* Infected Dogs

| Parameters | Control | 8 days PI | 16 days PI | 24 days PI |
|---|---|---|---|---|
| $QT_D$(sec) | 0.03±0.01 | 0.03±0.02 | 0.02±0.0 | 0.02±0.01 |
| $QT_{CD}$(sec) | 0.04±0.01 | 0.03±0.01 | 0.04±0.02 | 0.02±0.02 |
| MEA(˚) | 34.53±8.00 | 38.51±13.00 | 20.07±14.50 | 28.62±13.50 |
| K (mMoles/L) | 8.00±2.71 | 3.54±0.23* | 3.66±0.30* | 8.99±2.65* |

*Values are Mean ± SD, PI; post infection, QTD; QT dispersion, QTCD; heart rate corrected QT dispersion, MEA; mean electrical axis of the heart, *P<0.05 when compared to control group.*

*Table 2 :* Mean (± Sd) Of Plasma Potassium And Ecg Indices Of Dog With Or Without Arrythmia

| Parameters | Arrhythmic Dogs | Non-Arrhythmic Dogs | P value |
|---|---|---|---|
| Heart rate(bpm) | 172.87±26.43 | 154.80±30.76 | 0.084 |
| T/R wave voltage(mV) | 0.34±0.09 | 0.24±0.10 | 0.005 |
| $QT_D$(sec) | 0.02±0.01 | 0.03±0.02 | 0.029 |
| $QT_{CD}$(sec) | 0.02±0.01 | 0.04±0.01 | 0.003 |
| MEA(˚) | 12.28±25.79 | 9.00±29.03 | 0.736 |
| K ( mmol/L) | 7.19±3.40 | 5.37±2.76 | 0.109 |

*QTD; QT dispersion. QTCD; heart rate corrected QT dispersion. bpm; beats per minute, T/R; T voltage, MEA; Mean electrical axis of the heart.*

*Table 3 :* Variation Post Infection Of The Qt And Qtc Indices Of Lead Ii Electrocardiogram Of *T. Brucei* Infected Dogs

| ECG indices | control | 8 days PI | 16 days PI | 24 days PI |
|---|---|---|---|---|
| QT | 0.19±0.03 | 0.16±0.01* | 0.16±0.01* | 0.16±0.01* |
| $QT_C$ | 0.26±0.02 | 0.22±0.01* | 0.25±0.01 | 0.23±0.01* |

*Values are expressed as mean±SD; PI; post-infection and *p<0.05 compared to control.*

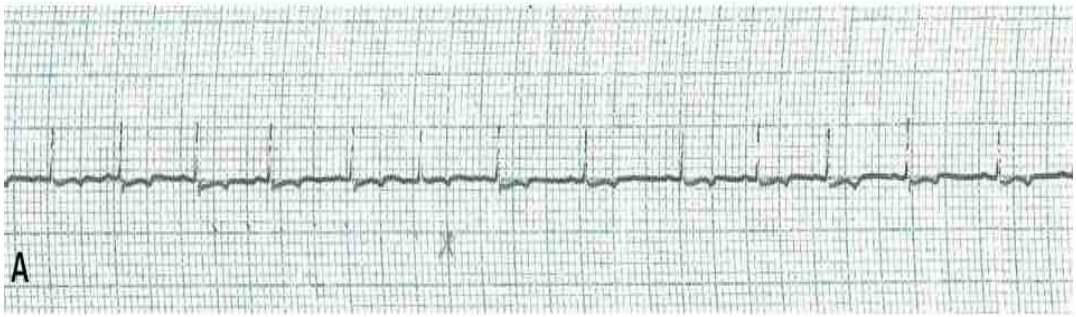


*Figure A :* lead II Electrocardiogram of uninfected control dogs showing normal sinus rhythm

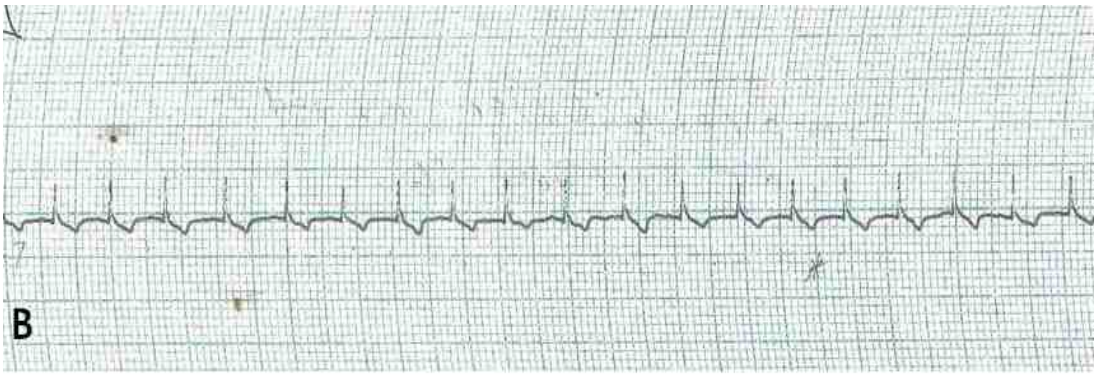


*Figure B :* Lead II Electrocardiogram of *T. brucei* infected dogs showing sinoventricular rhythms on the 8th day post infection. Note the absence of P waves

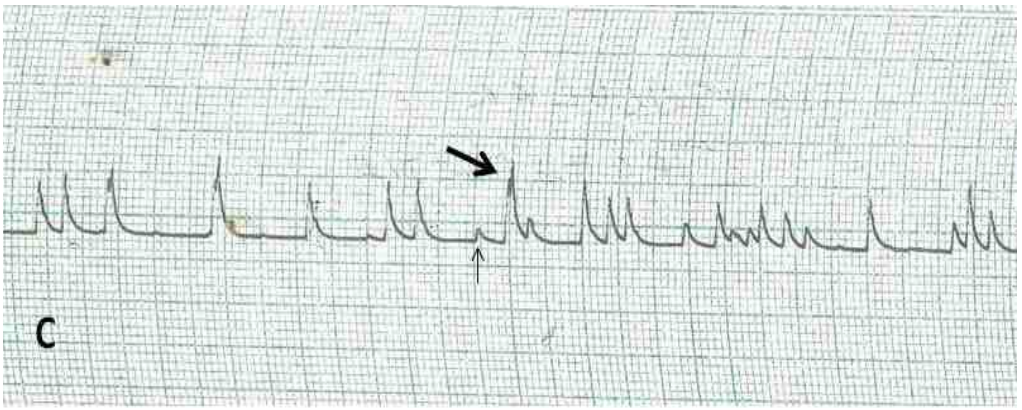


*Figure C :* Lead II Electrocardiogram of *T. brucei* infected dogs showing ventricular tachycardia on the 16th day post infection. Note the bizarre appearance of the notched R waves (thick arrow) and the P wave without a corresponding normal R wave

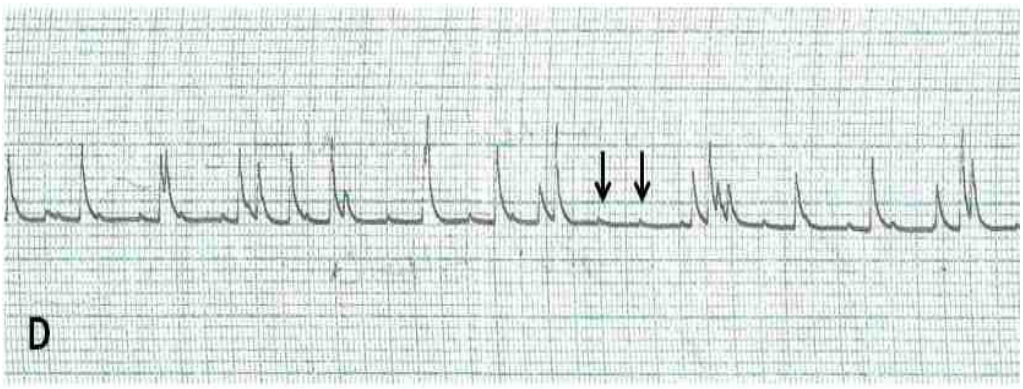


*Figure D :* Lead II Electrocardiogram of *T. brucei* infected dogs showing ventricular tachycardia and AV block on the 24th day post infection. Note the bizarre appearance of the R wave and the P waves (arrow) without a corresponding QRS complex

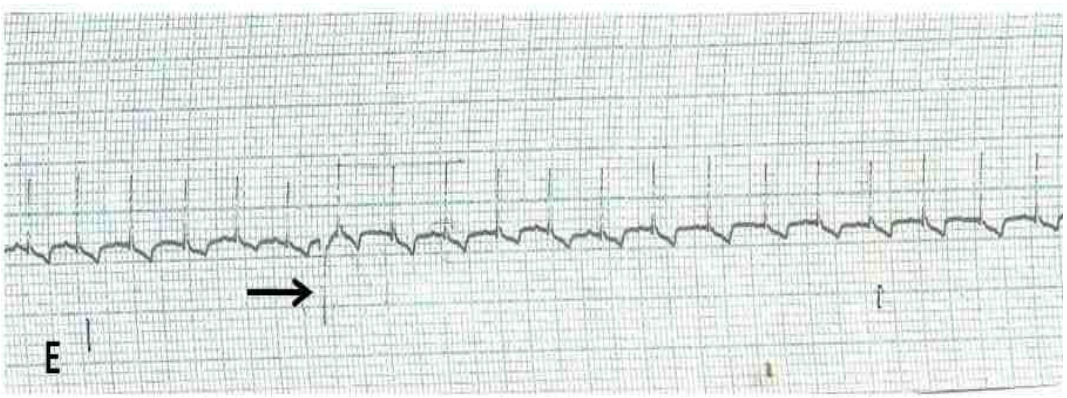


*Figure E :* Lead II Electrocardiogram of *T. brucei infected* dogs showing premature ventricular contraction. Note the negative polarity of the R wave

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# Effect of Sisal Foil Wrapped Milk Containers on Quality Parameters of Camel Milk Marketed in Borana Zone, Southern Ethiopia

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*Abstract-* The study was designed to evaluate effectiveness of Sisal foil wrapped milk containers on enhancing shelf life of the camel milk that was transported long distance in Borana zone. Hence, the primary lactodency meter test indicated the specific gravity of camel milk ranged from 1.020-1.022 at 20°c lactodency meter. At farm gate samples were negative for alcohol test that insured its freshness. All the samples in wrapped containers stayed negative for both alcohol and clot-on-boiling test at the terminal market, whereas, the rest were positive for alcohol test. Resazurin test revealed that the entire samples didn't show any significant variation in color change during the first 10min. After one hour of incubation, however, sample in new plastic container exposed to sun light was totally changed to pink followed by in local most exposed container that was changed to whitish pink only after 3hours of incubation. The sample in new plastic container that was most exposed to sun light cultured highest microbial load (6 x 105) followed by sample in local most exposed container (4 x 105) where as none of the sample in wrapped containers harbored significant load (4 x 105).

*Keywords: camel milk, marketed milk, milk container, milk quality, sisal foil and borana.*

*GJMR-G Classification : NLMC Code: WD 530, FOR Code: 839999p*


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# Effect of Sisal Foil Wrapped Milk Containers on Quality Parameters of Camel Milk Marketed in Borana Zone, Southern Ethiopia

Dejene Takele [α] & Tamiru Amanu [σ]

*Abstract-* The study was designed to evaluate effectiveness of Sisal foil wrapped milk containers on enhancing shelf life of the camel milk that was transported long distance in Borana zone. Hence, the primary lactodency meter test indicated the specific gravity of camel milk ranged from 1.020-1.022 at 20°c lactodency meter. At farm gate samples were negative for alcohol test that insured its freshness. All the samples in wrapped containers stayed negative for both alcohol and clot-on-boiling test at the terminal market, whereas, the rest were positive for alcohol test. Resazurin test revealed that the entire samples didn't show any significant variation in color change during the first 10min. After one hour of incubation, however, sample in new plastic container exposed to sun light was totally changed to pink followed by in local most exposed container that was changed to whitish pink only after 3hours of incubation. The sample in new plastic container that was most exposed to sun light cultured highest microbial load (6 x 105) followed by sample in local most exposed container (4 x 105) where as none of the sample in wrapped containers harbored significant load (4 x 105). The result of the study enabled us to conclude that wrapping the container has a paramount importance in maintaining the quality of milk transported long distance exposed to sun light. Hence, all the participants responsible for milk quality monitoring and enhancement have to be strengthened and scale up this technology.

*Keywords: camel milk, marketed milk, milk container, milk quality, sisal foil and borana.*

## I. Introduction

Milk is a marvel of nature and a very nutritious biological fluid which is produced by lactating animals to feed their offspring naturally. However, milk and milk products are indispensable components of the food chain of human being throughout the world. In most part of the world cattle milk is consumed much than other milk sources like Goats, camel, buffalo and sheep. Recently, because of its outstanding performance in the arid and semi-arid areas of south-east lowlands of Ethiopia where browse and water availability are limited, pastoralists rely mainly on camels for their livelihood (Bekele et al 2002). In these areas, camels are mainly kept for milk production and produce milk for a longer period of time even during the dry season when milk from cattle is scarce. In most pastoralists, camel milk is always consumed either fresh or in varying degrees of sourness of raw state without heat treatment and thus can pose a health hazard to the consumer.

Though it is dependent on genetics and environmental factors, camel milk is composed of much of water and other chemicals different in their composition. One of the parameters in camel milk quality is the accepted level of composition of these chemicals like the fatty acid, protein and lactose content, the pH level of the milk, and its test and texture. The milk quality can be affected at different levels starting from the physiology of the animals to be milked, and event of milking, collecting, transporting, processing and distribution of milk. The study area is characterized by lack of refrigeration facilities during milking and transportation. For instance the report of YONAD Business Promotion and Consultancy Service (2009) revealed that utilizing plastic containers for camel milk transportation from central Borana to Kenya border is the primary causes for milk quality deterioration since milk is highly perishable product. Therefore, having a due attention to total quality aspects of milk production and consumption; quality detection and safety precautions became of paramount importance. Thus, it was necessitated to develop refrigerating technology from locally available materials like wrapping milk containers by the foil that was obtained from the plant species so called sisal. Hence, this study was designed to evaluate the effectiveness of Sisal foil wrapped milk container that was soaked in water on reducing microbial growth and increased shelf life of the camel milk, transported long distance exposed to sunlight in Borana pastoral area.

## II. Material and Methods

### a) Study area description

The study was carried out in pastoral communities of southern Ethiopia, Borana Zone of Oromia Regional State during the dry and wet seasons of 2011 to 2012. The sites selected for the study purpose included Surupa kebele from Yabello district and Moyale town of Moyale district. Surupa kebele, the

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initial site, is located at about 50km North of Yabello town on the high way to Addis Ababa whereas Moyale town, the terminal site, is situated at 200km South of Yabello town at the border of Ethio-Kenya. Thus, the focus of the study was milk transported over 250 km distance from the above location to Moyale town.

*b) Methods of sample collection and laboratory analysis*

Samples of camel milk were taken, transported and analyzed following standard procedure (Richardson, 1985). Fresh morning camel milk samples were collected at farm level (Olla). Pastoralists were pre-informed to prepare, as possible as, clean unadulterated milk. All the milk samples collected from the pastoralists were tested for primary quality tests which included Specific gravity, Organoliptic test (smell, color and appearance of the milk), and Alcohol test). Those which were negative for the tests were considered as good quality milk and mixed to make homogenous milk before transferring to treatment containers for the initial quality test that was designed as:

T1= 4 Local Milk Containers (currently under utilization by the community)

T2= 4 Unwrapped New Plastic Milk Container

T3= 4 Wrapped New Plastic Milk Container

Except for the four local ones, those were fumigated and handled according to the community's indigenous knowledge; the remaining containers (wrapped and unwrapped new plastic containers) were sterilized using hot water. Variations in terms of where the containers had been placed on the vehicle were controlled as much as possible. Therefore, care was taken on how the milk containers were placed on the vehicle and deliberate efforts were made to ensure the containers were placed systematically every time in a repeatable way so that some received more air movement and sun light and others less. Thus, the above treatments were sub-divided as it was labeled below with regard to their placement pattern on the vehicle to conduct quality test at the terminal point:

- NMEC (Most Exposed Container)
- NLEC (New Less Exposed Containers)
- LMEC (Local Most Exposed Container)
- LLEC (Local Less Exposed Container)
- WMEC( Wrapped Most Exposed Container)
- WLEC (Wrapped Less Exposed Container)

Mixed and homogenized 1liter of milk sample was transferred to each of the container. Thermometer reading was taken from each container before transportation. Half of the containers (2 from each treatment) were kept on the upper layer of the entire container properly arranged and loaded to the car used for human transportation, in a way it was freely exposed to the sunlight. Whereas the remaining half from each treatment were loaded at the bottom of the layer of the container loaded on the car to prevent direct exposure to sun light and strong wind pressure. 100ml of samples representing the respective treatment was collected in well sealed bottles for utilizing as a control and kept under the refrigerator temperature in Ice box to be utilized as a control. The sample was immediately taken to the laboratory station of Yabello Pastoral and Dryland Agriculture Research Center.

*c) Terminal market (Moyale) milk quality*

At the terminal point where the milk is sold Plat form tests (Organoleptic, Alcohol and Clot on boiling) for each treatment sample was performed and temperature reading was also taken. For Further quality test, 100ml sample of milk from each treatment was collected and kept under refrigerator temperature in Ice box and brought to aforementioned laboratory station.

*d) Laboratory analysis*

i. *Titratable acidity*

The titratable acidity of all the samples (From the farm and terminal market) was determined by the quantity of a standard alkaline solution (0.1 N NaOH) which is required to neutralize the milk in the presence of phenophitaline.

ii. *The resazurin test*

Resazurin solution was prepared as per the standard procedure of one ml of the solution was placed in sterile test tubes then 10ml of the milk samples were added to each test tube. The samples were incubated at 37°C and result was recorded at 10min., 1hr.and 3hrs interval.

iii. *Microbial count*

Aerobic plate count was done within 12 hr of arrival of the samples at the laboratory. Enumeration of total aerobicmesophilic bacteria was done after plating 1 ml of the 10-5 dilution of the samples onto Standard Plate Count Agar. The agar plates were incubated aerobically at 35oC for 48 hr with replications. After incubation colony was count by counter and result was expressed as colony forming unit per one ml of milk (cfu/ml).

*e) Data analysis*

Descriptive statistics was utilized to compute the required data of the treatments, and the independent t-test was also employed to analyze the data of the treatment along the seasons of the area.

## III. Results and Discussion

*a) Quality of milk at producer (Olla) and terminal market*

Having all the experimental containers the team was arrived at village (Olla) from where the sample was

taken. The wrapped container just right wrapping was depicted in the picture below.


*Figure 1 :* Pictures of containers during wrapping at Yabello Pastoral and Dryland Agriculture Research Center, and arranged experimental containers for sample collection at Surupa kebele

It was identified that camel milk was pooled together by producers of the respective villages (Olla) of Borana zone to be availed for domestic market and/or Moyale town to be consumed by the dwellers of Ethiopian Moyale or crossed the border for Kenyan Moyale residents. Mode of transportation was that milk with plastic containers was solely loaded on the back of the vehicles for human transportation. There is also one Isuzu truck which transports 2500 liter milk (up to 250 Jerry cans with a volume of 10 liter) daily. The milk reaches to its destination in the afternoon between 1:00pm – 2:00pm. The following pictures depict the plastic jerry cans with collected milk that has been loading for transporting to Moyale town and backing the empty containers to Surupa PA.

*Figure 2 :* Systematically arranged containers for transporting camel milk from Surupa kebele to Moyale town and backing the empty containers to Surupa

The quality test for camel milk collected from producers (Olla) and after it reached a terminal market (Moyale) during both seasons of the area were conducted as presented in Table 1 and Table 2, respectively. The smell of milk both during the dry and wet seasons was smoky since all the pastoralists in the study area have been smoking their milk containers for various purposes (Table 1). For instance, smoking milk containers has been reported to exert anti-microbial properties and prolong the shelf life of milk (Ashenafi 1996). It was clearly observed from the physical derbies in the milk that pastoralists produce their milk under none hygienic environment. According to Abdurrahman (1995), poor management and unhygienic milking practices prevalent in the traditional husbandry systems, which include tying the teats with soft barks to prevent the calf from suckling, tick infestations and cauterization of the udder and skin, are few of the factors responsible for contamination of milk. There was specific gravity variation of camel milk during the dry and wet seasons of the study area probably due to moisture content difference along the seasons ($p < 0.01$). In this study it was observed that the specific gravity during the dry season ranged from 1.020-1.022 at 20°c calibrated lactodency meter (Table 1). However, it ranged from 0.995-1.002 at 20°c calibrated lactodency meter. At initial point (Olla) all the samples collected were negative for alcohol test that was evidence for no or very low production of acid at farm level which indicates the freshness of the milk. Significant temperature variation

was observed ($p<0.01$) for the milk in the wrapped and unwrapped containers mainly due to the unwrapped containers' absorption of the environmental temperature. Similarly, there was also variation in temperature of milk in unwrapped containers those were labeled as NC and LC, during the two seasons of the study area. That might be due to the fact that environmental temperature of wet season was cooler than dry season, particularly in the morning while we collected the sample. The relatively lesser temperature rise for wrapped container that was labeled as WNC during wet season was principally because of the cooling nature of wrapping.

The rise in temperature was relatively lower for wrapped containers and during the wet season as well (Table 1). It was possible to observe that the samples with higher temperature were positive for alcohol and clot-on-boiling test (Table 1 and Table 2). This result is in line with the report of 0'Connor (1995) which states that temperature is the most determining factor for milk fermentation and hence quality deterioration.

*Table1 :* Primary quality tests of camel milk at producers (Olla) during dry and wet seasons of Borana zone

| Sample No | Dry season | | | Wet season | | | Dry season | | Wet season | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Smell | Color | Appearance | Smell | Color | Appearance | Specific gravity | Alcohol (68%) | Specific gravity | Alcohol (68%) |
| 1 | Smoked | Yellowish white | Physical Derbies | Smoked | Whitish | Physical Derbies | 1.020 | -Ve | 0.995 | -Ve |
| 2 | Smoked | Yellowish white | Physical Derbies | Smoked | Whitish | Physical Derbies | 1.021 | -Ve | 0.997 | -Ve |
| 3 | Smoked | Yellowish white | Physical Derbies | Smoked | Whitish | Physical Derbies | 1.022 | -Ve | 1.002 | -Ve |
| 4 | Smoked | Yellowish white | Physical Derbies | Smoked | Whitish | Physical Derbies | 1.021 | -Ve | 1.001 | -Ve |
| 5 | Smoked | Yellowish white | Physical Derbies | Smoked | Whitish | Physical Derbies | 1.020 | -Ve | 1.001 | -Ve |

*Temperature of pooled sample of camel milk within the three treatments at the initial point (Olla) during the dryand wet seasons*

| Type of Container | Code for Container | Dry season(26°C[2]) | Wet season(21°C[1]) |
|---|---|---|---|
| Wrapped New Container | WNC | 21°C | 19°C |
| Unwrapped New Container | NC | 24°C | 20°C |
| Local Container | LC | 25°C | 24°C |

Test for various Organoleptic and temperature measurement of milk at the terminal market (Moyale town) revealed that there was some similarity and discrepancy for wrapping and not wrapping the containers. The discrepancy also held for the seasons of the study site as summarized in Table 2. All the wrapped and soaked containers stayed negative for both alcohol and clot on boiling test at the terminal milk market during both seasons (Table 2). At the terminal market soaked containers relatively stayed cool than unsoaked containers. The exposed milk containers had significantly higher temperature than less exposed containers ($p<0.01$).

*Table 2 :* Primary quality tests of camel milk at terminal point (Moyale town) during the dry and wet seasons of Borana zone

| Sample code | Dry Season(30°C*) | | | | | | Wet Season (25°C[2]) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Alcohol Test | Clot on boiling | Smell | Color | Appearance | Milk Temperature | Alcohol Test | Clot on boiling | Smell | Color | Appearance | Milk Temperature |
| WMEC | -ve | -ve | Smoked | Yellowish white | Physical derbies | 27°C | -ve | -ve | Smoked | Whitish | Physical derbies | 24°C |
| WLEC | -ve | -ve | Smoked | Yellowish white | Physical derbies | 25°C | -ve | -ve | Smoked | Whitish | Physical derbies | 23°C |
| LMEC | Turbid/ Sediment | +ve | Smoked | Yellowish white | Physical derbies &Minor curdling | 33°C | Turbid/ Sediment | +ve | Smoked | Whitish | Physical derbies | 28°C |

[1] Indicates the environmental temperature of the study area (At initial point)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LLEC | Sediment | +ve | Smoked | Yellowish white | Physical derbies | 30°C | -ve | +ve | Smoked | Whitish | Physical derbies | 26°C |
| NMEC | Clear sedimentation | +ve | Smoked | Yellowish white | Viscous | 34°C | Turbid/ Sediment | +ve | Smoked | Whitish | Physical derbies &Minor curdling | 30°C |
| NLEC | Sediment | +ve | Smoked | Yellowish white | Viscous | 30°C | Turbid/ Sediment | +ve | Smoked | Whitish | Physical derbies &Minor curdling | 28°C |

*WMEC: Wrapped container exposed to Sun light; WLEC: Wrapped container less exposed to Sunlight; LMEC: Local container exposed to sunlight; LLEC: Local container less exposed to sunlight; NMEC: New container exposed to sunlight; NLEC: New container less exposed to sunlight.*

Milk of other treatments with unwrapped and unsoaked containers was remained positive for alcohol and clot-on-boiling test. That might due to the development of lactic acid from milk fermentation because of exposure the containers to sun light. The result was proved according to the report of 0'Connor (1995) which states that alcohol test is an alternative method of measuring the acid accumulation of milk since it is more sensitive for acid than clot-on-boiling test.

*b) Laboratory sample analysis result*

i. *Titratable acidity*

The acidity value of samples from terminal site (Moyale town) during both seasons was evaluated at N° (0.1 NaOH) as summarized in Table 3.

*Table 3 :* Titratable acidity value of camel milk from terminal site during the dry and wet season of Borana zone

| Sample code | Dry Season | | Wet Season | |
|---|---|---|---|---|
| | N° (0.1 NaOH) | Lactic acid | N° (0.1 NaOH) | Lactic acid |
| WMEC | 2.30 | 0.230 | 2.28 | 0.228 |
| WLEC | 2.28 | 0.228 | 2.27 | 0.227 |
| LMEC | 2.38 | 0.238 | 2.35 | 0.235 |
| LLEC | 2.35 | 0.235 | 2.32 | 0.232 |
| NMEC | 2.40 | 0.240 | 2.36 | 0.236 |
| NLEC | 2.36 | 0.236 | 2.33 | 0.233 |
| Control | 2.25 | 0.225 | 2.25 | 0.225 |

*WMEC: Wrapped container exposed to Sun light; WLEC: Wrapped container less exposed to Sunlight; LMEC: Local container exposed to sunlight; LLEC: Local container less exposed to sunlight; NMEC: New container exposed to sunlight; NLEC: New container less exposed to sunlight.*

The lactic acid secretion of milk in the wrapped containers was relatively lower than unwrapped during both seasons of the study site. On the other hand, the containers with no wrapping stimulated the milk to produce extra lactic acid which strongly deteriorates the quality parameters. The secretion of lactic acid during the wet season was significantly lower than the dry season ($p<0.01$). The same was true for less exposed containers than the most exposed ones. The results were in line with the report of T. Ahmed and R. Kanwal (2004) which states that when camel milk is left to stand and heated moderately, the acidity rapidly increases due to the presence of lactic acid producing bacteria.

ii. *Resazurin test*

The dye reduction value of the whole representative sample with three time interval was only analyzed for dry season due to chemical constraint the researcher faced to repeat during the wet season of the study area. The milk samples didn't show any significant variation in color change during the first 10min whereas after an hour the new plastic container that was exposed to sun light was totally changed to pink (Table 4).

*Table 4 :* Resazurin test of camel milk from terminal site during the dry season of Borana zone

| Sample Code | 10min. | 1hr. | 3hr. |
|---|---|---|---|
| WME | Light purple | Light purple | Light purple |
| WLE | Light purple | Light purple | Light purple |
| LME | Light purple | Purple pink | Whitish pink |
| LLE | Light purple | Light purple | Pink |
| NME | Light purple | Pink | White |
| NLE | Light purple | Slightly Purple pink | Pink |
| Control | Light purple | Light purple | Light purple |

After 1hr the samples in unwrapped new containers as well as in local containers there were color change which is an indication of becoming poor in quality. Even after 3hrs incubation the samples in wrapped containers remained unchanged. In the contrary the dye was totally reduced in the sample from new plastic container that was exposed to sun light that showed bad quality milk. While, the local containers that

[2] Indicates environmental temperature of the study area

was most exposed to sun light was changed to whitish pink after 3hr of incubation. Whereas the local containers were in better position than new plastic containers this might be due to the fact that the containers were well smoked. Compounds released from smoking wooden trees namely Olea africana (Egeresa) and Balanites galbara during smoking of the containers may be responsible for the longer shelf life of camel milk (Eyassu, 2007).

iii. *Total microbial load*

The average microbial count for the samples of camel milk under different containers of the treatments was undertaken for dry season of study area despite not done for the wet season due to the laboratory equipment damage during that season. The researchers did not differentiated the micro-organisms were economically important or not than counting the load. The result showed that the milk samples kept in new plastic containers those were most exposed to sun light had the highest microbial load (6 x 105) followed by the local containers those were most exposed (4 x 105). Whereas wrapped containers had a positive effect on maintaining good quality of milk during transportation. The microbial load difference might be associated with post harvest handling. For instance, at bulking and market centers, microbial contamination increased to almost 100% cfu/ml for the camel milk being stored at high temperature on transit to other distant markets from farm environment (Matofari J. W., et al, 2013).

*Table 5 :* Microbial count under the different treatments of dry season of Borana zone

| *Sample Code* | *Colony Forming Unit (CFU/ml of milk* |
|---|---|
| *WME* | $2.0 \times 10^4$ |
| *WLE* | $2.0 \times 10^4$ |
| *LME* | $4.0 \times 10^5$ |
| *LLE* | $3.0 \times 10^4$ |
| *NME* | $6.0 \times 10^5$ |
| *NLE* | $1.5 \times 10^5$ |
| *Control* | $1.0 \times 10^4$ |

## IV. Conclusion and Recommendations

The result of the study enabled us to generally conclude that wrapping the containers has an importance in maintaining the quality of milk transported long distance exposing to sun light. On the other hand wrapping containers has a great contribution in minimizing microbial load and lactic acid production as of the fermentation. Hence, the stakeholders responsible for milk quality monitoring and enhancement have to be strengthened for scaling up this technology since it is found to be effective in maintaining the quality of milk involved in market being transported long distance.

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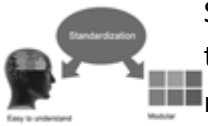


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The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.


After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

**The following entitlements are applicable to individual Fellows:**


Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

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We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

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**The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:**

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
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- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

**Note :**

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

# Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

**(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.**

**(II) Choose corresponding Journal.**

**(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.**

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# Preferred Author Guidelines

**MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)**

Page Size: 8.27" X 11'"

- Left Margin: 0.65
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- Top Margin: 0.75
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- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

**You can use your own standard format also.**
**Author Guidelines:**

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

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All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

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Complete support for both authors and co-author is provided.

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Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

**5.STRUCTURE AND FORMAT OF MANUSCRIPT**

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

**Papers**: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

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(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

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Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

**Format**

*Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.*

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Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

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Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

*Acknowledgements: Please make these as concise as possible.*

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

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*Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.*

*Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.*

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Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

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www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

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## TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

**1. Choosing the topic:** In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

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**3. Think Like Evaluators:** If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

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**5. Ask your Guides:** If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

**6. Use of computer is recommended:** As you are doing research in the field of Computer Science, then this point is quite obvious.

**7. Use right software:** Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

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**9. Use and get big pictures:** Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

**10. Bookmarks are useful:** When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

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**14. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

**15. Use of direct quotes:** When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

**16. Use proper verb tense:** Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

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**18. Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

**19. Know what you know:** Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

**20. Use good quality grammar:** Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

**21. Arrangement of information:** Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

**22. Never start in last minute:** Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

**23. Multitasking in research is not good:** Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

**24. Never copy others' work:** Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

**25. Take proper rest and food:** No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

**26. Go for seminars:** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

**27. Refresh your mind after intervals:** Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

**28. Make colleagues:** Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

**29. Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

**30. Think and then print:** When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

**31. Adding unnecessary information:** Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

**32. Never oversimplify everything:** To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

**33. Report concluded results:** Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

**34. After conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

## Informal Guidelines of Research Paper Writing

**Key points to remember:**

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

**Final Points:**

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

**Title Page:**

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

**Abstract:**

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

**Introduction:**

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

**Procedures (Methods and Materials):**

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

**Results:**

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

**Discussion:**

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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| Topics | Grades | | |
|---|---|---|---|
| | **A-B** | **C-D** | **E-F** |
| ***Abstract*** | Clear and concise with appropriate content, Correct format. 200 words or below | Unclear summary and no specific data, Incorrect form<br><br>Above 200 words | No specific data with ambiguous information<br><br>Above 250 words |
| ***Introduction*** | Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited | Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter | Out of place depth and content, hazy format |
| ***Methods and Procedures*** | Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads | Difficult to comprehend with embarrassed text, too much explanation but completed | Incorrect and unorganized structure with hazy meaning |
| ***Result*** | Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake | Complete and embarrassed text, difficult to comprehend | Irregular format with wrong facts and figures |
| ***Discussion*** | Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited | Wordy, unclear conclusion, spurious | Conclusion is not cited, unorganized, difficult to comprehend |
| ***References*** | Complete and correct format, well organized | Beside the point, Incomplete | Wrong format and structuring |

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