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Include volume of blood collected, container used, and amount of preservative or anticoagulant (e.g., heparin).

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The results are presented in the form of tables or figures when feasible. The text should explain or elaborate on the tabular data, but numbers should not be repeated within the text. Sufficient data, all with some index of variation attached (including significance level; i.e., *P*-value), should be presented to allow the reader to interpret the results of the experiment. Reporting the actual *P*-value is preferred to the use of the terms *significant* and *highly significant*. Thus, the observed significance level (e.g., $P = 0.027$) should be presented, thereby allowing the reader to decide what to reject. Other probability (alpha) levels may be discussed if properly qualified so that the reader is not misled (e.g., trends in the data).

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Productivity and Tonic Immobility Duration of Thai Crossbred Chickens Raised at Different Stocking Densities

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ABSTRACT

The objective of this study was to investigate the effects of stocking density (8, 12 and 16 birds/m2) on productivity and tonic immobility duration (a measure of fearfulness) of Thai crossbred chickens (n=900 birds) kept at 100 birds per pen. The results showed that stocking density had no significant (P>0.05) effect on body weight, body weight gain, feed intake, feed conversion ratio and mortality of chickens from the wk2 to 12. When stocking density was increased from 8 birds/m2 to 16 birds/m2, tonic immobility (TI) duration of the chickens increased significantly (P<0.05).However, the TI duration of chickens at a density of 12 birds/m2 was not significantly different from those of both the lower and the higher densities. In conclusion, Thai crossbred chickens could be stocked up to 12 birds/m^2 without adverse effect on productivity and welfare when compared to those kept at 8 birds/m^2.

Key words: Thai crossbred chicken, stocking density, productivity, tonic immobility

INTRODUCTION

Meat of native chickens is preferred by Thai people over the same products from commercial poultry because of their taste, leanness, and suitability to Thai special dishes (Wattanachant*et al.*, 2004). Thus, native chicken meat is more highly valued than that coming from commercial poultry. The domestic market for Thai native chickens has increased significantly and overseas markets also have strong potential. This has led to a change of practice in raising native chickens in Thailand. Cross breeding of Thai native males with egg type females, rather than pure breeding of Thai native chickens, is used to obtain higher chick production. It is recommended by the Department of Livestock Development, Thailand, that

stocking density used for open houses should be 8birds/m^2.However, some producers rear their chickens at higher stocking densities in order to reduce the fixed costs of production and produce more kilograms of chickens per unit area. As it is known the at reduction in space per bird generally results in poorer productivity and welfare of the chickens (Estevez, 2007).The objective of this study was to investigate the effect of rearing at higher than recommended stocking density on production and tonic immobility duration, a measure of fearfulness (Marin *et al*., 2001), in Thai crossbred chickens.

MATERIALS AND METHODS

A total of 900mixed sex Thai crossbred chicks(Thai native males and ISA Brown commercial layer type females), supplied by Suranaree University of Technology poultry farm (Thailand), were reared from one day old to 13 wk of age without the use of beak trimming. The experiment lasted from February to April, 2011.

The pen sizes were 12.5 m^2, 8.33 m^2, and 6.25 m^2 in area. There were 100 birds per pen. This resulted in treatment densities of 8, 12 and 16 birds/m^2, respectively. The pens were bedded with approximately 5 cm of rice hulls.

Chicks were brooded for 2 wk before being randomly assigned to the treatments. At the end of the second wk, the chicks were vaccinated according to the recommendation of the Department of Livestock Development, Thailand. The chickens were fed a standard commercial three phase broiler diet. Feed and water were fed ad libitum throughout the rearing period. During the first 3wk, feed was added 3 to 4 times a day. After that the feed was added twice per day (0800 h and 1630 h). The ratio of birds per feeder cup (diameter×high: 40 cm×30 cm) or water bottle (4L capacity) was 25 to one.

Natural lighting was used after the brooding period until 13 wk old. The chicken house was protected from the wind and rain with plastic sheeting, which was also used to adjust the ventilation. Before stocking the birds, the house was sprayed with disinfectant. Temperature and relative humidity in the chicken house were recorded continuously.

Data on average body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) and mortality rate were determined at the end of the experimentwhen they chickens were 12 wk old.

During wk 13 (from 85 to 88 d old), 7 birds, randomly chosen from each pen, were evaluated in the tonic immobility (TI) testin a separate area of the chicken house. TI was induced as soon as the bird was caught by placing the animal on its back, with the head hanging, in a V-shaped plastic cradle (length×width×height: 30×24×20 cm). The method was similar to that described by Campo *et al.* (2008). The bird was restrained for 10 s. The observer sat in full view of the bird, about 1 m away, and fixed his eyes on the bird to cause the fear-inducing properties of eye contact. If the bird remained immobile for 10 s after the researcher removed his hands, a stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in less than 10 s, and the restraint procedure was repeated (3 times maximum), then it was considered that tonic immobility had not been induced, so a 0 s score was given. If the bird did not show a righting response over the 10 min test period, a maximum score of 600 s was given for righting time.

The experimental unit considered was the pen. The experimental design used was a completely randomized design with three replicates per treatment. The data were subjected to analysis of variance with the General Linear Model procedure of SPSS 16.0. TI duration data were logarithmically transformed prior to analysis. When significance was indicated, differences among treatment means were tested by Duncan’s multiple range tests.

RESULTS

During the experiment, average temperature and relative humidity in the chicken house in the morning (0700 h) and the afternoon (1430 h) were (Means ± SE) 24.06 ± 0.29°C, 30.30 ± 0.46°C, 88.09 ± 0.99% and 67.05 ± 1.46%, respectively. Different levels of stocking density did not affect BW, BWG, FI, FCR or mortality (Table 1).

Table 1 Effects of stocking density on body weight (BW), body weight gain (BWG), feed intake (FI)and mortality of Thai crossbred chickens(Means ± SE)

Density(Birds/m^2)	BW(g)	BWG(g)	Feed Intake (g)	FCR	Mortality (%)
8	1293.30±43.33	1187.70±43.67	3367.60±66.67	2.85±0.14	1.67±0.02
12	1242.20±70.35	1137.60±67.57	3348.20±54.54	2.96±0.14	1.00±0.01
16	1275.00±72.86	1164.50±73.28	3423.90±26.51	2.95±0.17	0.33±0.01

Stocking density affected TI duration of the chickens (Table 2). The TI duration of chickensat16 birds/m^2 was higher ($P<0.05$) than that for 8 birds/m^2, while that at12 birds/m^2 density was not significantly different from either the higher or lower densities.

Table 2: Effects of stocking density on TI duration of Thai crossbred chickens

Density (Birds/m^2)	TI duration(s)
8	$284±48^{a}$
12	$327±48^{ab}$
16	$432±45^{b}$

^{a,b}means within the same column with different superscripts were significantly different ($P<0.05$)

DISCUSSION

Temperature and relative humidity recorded during the experiment were normal for Thailand and did not cause any adverse effects on the chickens. The final BW of the chickens (at 12 wk of age) was sufficient to reach the marketable live weight of 1.2 kg which is normal for Thai chickens (Haitook *et al*., 2003).

The results of this experiment agreed with those of Feddes *et al*. (2002) and Ravindran *et al*. (2006) who reported similar BW and BWG for chickens reared at three levels of low, middle, and high densities. The results also agreed with those of Thomas *et al*. (2004) who reported that stocking density had no effect on broiler mortality. However, Hall (2001) reported a significant increase of mortality in high stocking density in commercial farms. Dawkins *et al*. (2004) and Jones *et al*. (2005) argued that stocking density itself was less important to the physical health and mortality rates of the chickens than other environmental factors. Dawkins *et al*. (2004) showed that the differences between producers in terms of the environment they provide to the animals had more impact on their welfare than stocking density per se.

The longer TI duration observed at the highest stocking density indicates that the chickens were more fearful. These results are similar to the findings of Andrews *et al*. (1997) and Onbaşılar *et al*. (2008).The duration of TI response to manual restraint is widely considered to be a useful behavioral index of fear and thus welfare (Marin *et al*., 2001).This indicates that raising Thai cross breed chickens at 16 birds/m^2 can compromise one measure of chickens' welfare when compared to those raised at 8 birds/m^2.

In conclusion, the results of this experiment suggest that Thai crossbred chickens could be kept at a stocking density of 12 birds/$m^2$and maintain the same level of productivity and welfare status as those kept at the suggested 8 birds/m^2 by the Department of Livestock Development, Thailand.

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Nutrient Composition and In Vitro Gas Production of False Yam (*Icacinaoliviformis*) Leaves

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ABSTRACT

*Icacinaoliviformis*is common shrub found in the Northern Regions of Ghana. It has received very little attention regarding its use as feed supplement for ruminants. This study was carried out to determine the chemical composition and potential digestibility of the leaves of *Icacinaoliviformis* (IOL) when incubated with or without ammonium hydrogen carbonate (NH_4HCO_3) in the media. The media with (NH_4HCO_3) was referred to as nitrogen sufficient (NS) and the one without (NH_4HCO3) was nitrogen deficient (ND). The results of the study showed that IOL had a dry matter content of 377.3g/Kg with a crude protein content of 173g/kg. The NDF, ADF and ADL were 439.5, 393.5 and 191.9g/kg respectively. The gross energy content was estimated to be 18.4MJ/Kg DM. There was no significant difference ($p>0.05$) between the ND and NS media for all the parameters measured except for ammonium nitrogen where the NS was significantly ($p=0.021$) higher than the ND. The potential degradability recorded for the IOL in both ND and NS media was 158.47 and 170.93 respectively. The rate of degradability of the ND was 0.055 and that of NS was 0.043. The pH and ammonium nitrogen recorded were all within the optimum range required for microbial cell synthesis and cellulolysis. The IOL could be included in the feed formulation for ruminants in Ghana.

Keywords: *Icacinaoliviformis*, *in vitro* gas, leaves, nitrogen deficient, nitrogen sufficient.

INTRODUCTION

Icacinaoliviformis is an underutilised shrub found in the savannah regions of Ghana. It is a drought tolerant plant and usually produces fresh leaves especially in the dry season when most conventional sources of forage are dry and of poor nutrient value. The plant has a huge tuber beneath and is sometimes referred to as false yam. The tuber sometimes weighs over 50kg and provides a rich source of starch in times of famine (Fay, 1987). The tuber has been

reported to contain protein and carbohydrate contents of 5.4% and 53.1% respectively (Dei *et al.*, 2011).The use of *I.oliviformis* in diet of human has been limited by the presence of a gum resin which makes up 0.9-2.8% of the tuber (NRI, 1987). The leaves of *Icacinaoliviformish* as not been investigated for its potential as feed for ruminant since the plant was discovered. The presence of plant secondary metabolites (PSM) in trees and shrubs of tropical origin makes the nitrogen in them insufficient for microbial degradation (Salem, 2005). This insufficient supply of nitrogen in the rumen has been attributed to the complex formed between PSM especially tannins and the dietary protein at a pH of 4.0. To 7.0 in the rumen (Osuga, *et al*., 2005; Min *et al*., 2005). Ansah *et al*. (2012) included the leaves in the diet of rabbits at 5% and 10 inclusion levels. The results of that study showed a better growth and digestibility from the animals on the 5% diet. The present study sought to investigate the potential digestibility and chemical composition of the *Icacinaoliviformis* leaves.

Hypothesis

Excluding nitrogen from the in vitro incubation media will not reduce the in vitro gas production (IVGP) of *Icacinaoliviformis* significantly.

MATERIALS AND METHODS

Study Area

Fresh leaves of *Icacinaoliviformis* (IOL) were harvested around the Nyankpala campus of the University for Development Studies during the early dry season (October to November, 2011). The leaves were then shade dried and milled to pass through 1mm sieve screen and packaged into plastic bags, sealed and transported to the Princes Margret laboratory of the Harper Adams University for nutrient analysis and in vitro gas studies.

Chemical Analysis

Fresh IOL samples (200.0g) were placed in an oven set to a temperature of 60^0C for a period of 48hours after the initial weight was recorded (AOAC, 2000). The new weight after drying was used to calculate the dry matter content of the samples and expressed in g/kg. The dry matter was determined at the Agriculture Sub-sector Improvement Project (AgSsIP) laboratory at the Nyankpala Campus of the University for Development Studies.

Shade dried milled IOL samples were analyzed for nitrogen and used for computing the crude protein content using the formulae Crude protein (g/kg DM) = total nitrogen (g/kg DM) x 6.25. The nitrogen content was determined by combusting 1.0g of each sample with oxygen using the Leco (FP-528-UK).

The nitrogen oxide after combustion is reduced to nitrogen and measured with a thermal conductivity detector with helium as a reference. Ash content was determined by burning 2.0g of the samples at a temperature of 550^0C for 4hours in a mufflefurnace (Carbolite, AAF 1100, Hope valley, England). The residue was weighed and used for computing the ash (AOAC, 2000). The ash content was subtracted from the dry matter content to get the organic matter content.

The ether content was extracted using petroleum ether (AOAC, 2000). The gross energy content was determined by combusting 2.0g of each sample using the bomb calorimeter (Parr 6200 Calorimeter-UK).The neutral detergent fiber, acid detergent fber and acid detergent lignin were analyzed according to the method of Goering and Van Soest (1970).

All chemical analyses were done at the nutrition laboratory of the *Princess Margaret Laboratories* of the Harper Adams University.

In vitro gas analysis and experimental design

The randomized complete block design was used with each treatment having 5 replicates. The method described by the odorou *et al*. (1991) was used for the *In vitro* gas studies. In this particular study, the nitrogen content of the media was varied in a way that in one media, ammonium hydrogen carbonate (NH_4HCO_3) was excluded to represent the nitrogen deficient media (ND) and the other media had ammonium hydrogen carbonate (NH_4HCO_3) representing the nitrogen sufficient media (NS). The NS media preparation was done according to the procedure of the odorou *et al.* (1991) and the ND was done according to the procedure of Getachew *et al.* (2000). Approximately 2.0g of IOL was weighed into a 250ml fischer and duran bottles and placed in an incubator (39^0C) overnight.

Rumen fluid was collected from 4 rams fitted with a fistula. The animals had an average weight of 95kg. They were fed *ad libitum* on wheat straw and offered 250 g/day of concentrate (Wynnstay ram master coarse mix, UK) at a rate of 1.1 x maintenance (AFRC 1993). The rams were group housed with straw as bedding and under constant light supply. Water was offered *ad libitum*.

Rumen fluid was collected 3-4hours post feeding through a suction process. The fluid was collected into a pre-warmed vacuum flask and quickly sent to the laboratory. The fluid from each of the four animals were pooled and strained using a four layer cheese cloth placed over a 5 liter conical flask with help of a funnel.

On the day of incubation the two media were placed in a water bath with the temperature set to 39^0C. Carbon dioxide was continuously flushed through each media to ensure they are anaerobic. The strained rumen fluid was mixed with media in a proportion of 1.4 liters rumen fluid to 10 liters media (Huntington *et al*., 1998). The rumen fluid formed 14% of the total incubation buffered rumen fluid.

Approximately 200ml of the buffered rumen fluid (Media + rumen fluid) was pumped into the pre-weighed samples in the vessels using a peristaltic pump, gassed for 5seconds, sealed with rubber corks and placed in the incubator set to 39^0C. The rubber corks were fitted with a needle and valve to aid in the measuring of pressure (gas accumulation).

Gas production from each vessel was measured using a pressure transducer. The pressure readings were taken at 0, 3, 6, 12, 18, 24, 36, 48, 60, 72 hours and converted from PSI to volume (ml/g DM). The pressure reading at time zero was assumed to be zero since there is no method available to measure gas production at time zero.

After each incubation period, the pH of the content of each bottle was measured. The content of each bottle was strained using a four layer cheese cloth. The liquid was stored for analysis of ammonium nitrogen.

Exactly 5ml of defrosted filtrate from the in vitro experiment was measured into digestion tube and 6ml of magnesium oxide added. The sample was steamed via a receiver solution prepared from 50g boric acid, 50ml bromocresol green and 35ml methyl red in 5L distilled water. This was back titrated using 5mM sulphuric acid to determine the color change. Ammonium nitrogen was calculated from the titer value obtained for each sample using the following formulae:

$$\textbf{Ammonium nitrogen (g/L)} = \frac{\textit{Sample titre (ml)}}{\textit{Weight of sample distilled (g)}} \times \mathbf{0.01401} \times \mathbf{0.01} \times \mathbf{1000}$$

Where 0.01401 represents the weight of nitrogen atom and 0.01 is the concentration (normality) of the acid solution.

Statistical Analysis

The gas measurements were then fitted to the exponential curve of Orskov and McDonald (1979) without an intercept using sigmaPlot 10th edition.

$$Y = b (1 - e^{-ct})$$

Where:
Y = gas volume at time t (ml)
b = asymptotic gas production (ml/g DM)
t = time (h)
c = fractional rate of gas production (ml/h)

The data was analyzed using the independent t-test in statistical package for social sciences (SPSS) 18th edition. The in vitro production curves were plotted using MS Excel, 2010.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of the *Icacinaoliviformis* leaf (IOL). The CP obtained was higher than what was reported by Dei *et al*. (2011) for *Icacinaoliviformis* tuber. The crude protein in IOL was above the 70g/kg DM required to enhance voluntary feed intake in ruminant (Nori *et al.*, 2009).

Table 1. Chemical composition of *Icacinaoliviformis* leaf

Composition	g/kg
DM	377.3±4.4
Ash	68.9±0.9
OM	308.4±0.5
CP	173.0±1.3
Ether	39.3±1.6
NDF	439.5±2.1
ADF	393.5±5.4
ADL	191.9±3.7
GE MJ/kg DM	18.4±0.2

The CP reported for the IOL was higher than what has been reported for most grasses and cereal based crop residue which serve as the main source of forage for free ranging ruminant in the dry season. Alhassan *et al*. (1999) reported that the protein content of most grasses drop from an average of 12.5% in the wet season to 3% in the dry season. Avornyo*et al*. (2000) reported a crude protein content of 3.3% for rice straw which is a common cereal based crop residue eaten by ruminant in the dry season. The neutral detergent fiber (NDF) and acid detergent fber (ADF) compared favourably with the values reported for some tropical legume shrubs (Zhou *et al*., 2011; McSweeny *et al*., 2009).

Effects of additional nitrogen on fermentation pattern

Results on the effect of additional nitrogen on the *in vitro* gas production (IVGP) are presented in Table 2. There was no significant difference between the 2 media on potential degradability (P=0.570), rate of degradability (P=0.156) and pH (P=0.764). There was a significant difference (P=0.021) between the two treatments for ammonium nitrogen.

Table 2. Effect of additional nitrogen on *in vitro* rumen degradability and ammonium nitrogen concentration of IOL

Media	C	a+b	pH	NH_4N (mg/l)
ND	0.055	158.47	6.74	268
NS	0.043	170.93	6.82	321
Sed	*0.074*	*20.77*	*0.248*	*0.174*
P.value	*0.156*	*0.570*	*0.764*	*0.021*

C=rate of degradability, a+b =potential degradability

The nitrogen sufficient (NS) media had a higher potential degradability than the nitrogen deficient (ND). Ammonium nitrogen of the incubation media was higher for the NS compared with the ND. The gas production for IOL in both ND and NS witnessed a sharp increase within the first 12hours. After 12hours, IOL in the NS media declined marginally and began to increase after 24hours (Fig1).

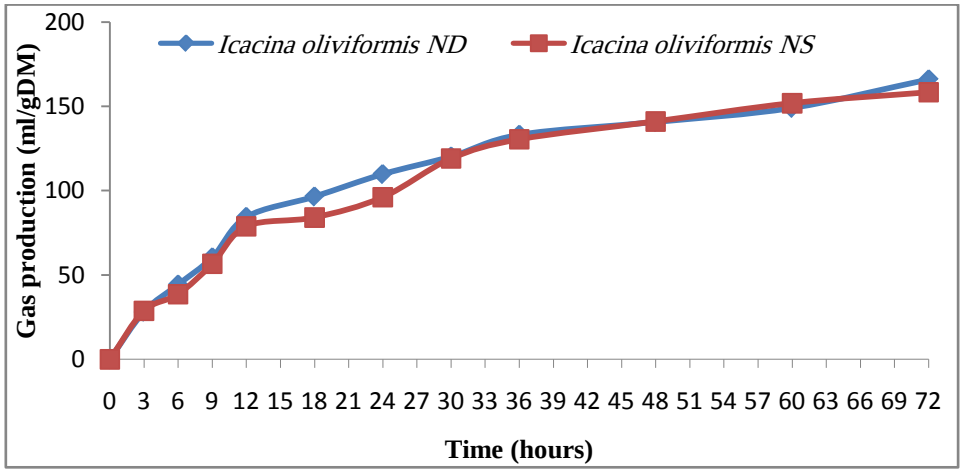

Figure 1. Cumulative *in vitro* gas production of *Icacinaoliviformis* leaf in nitrogen deficient and nitrogen sufficient media

Ammonium nitrogen concentration in the incubation media is a balance between degradation of dietary protein and the uptake of ammonium nitrogen for microbial cell synthesis (Hariadi and Santoso, 2009). The ammonium nitrogen recorded in the ND media could be due to the microbial degradation of the protein in the IOL substrate. Fiber degrading microbes in the rumen require ammonium nitrogen for cell synthesis in order to multiply and degrade the fiber to supply the volatile fatty acids for absorption (Russell *et al.*, 1992). Satter and Slyter (1974) reported that ruminal ammonia concentrations between 88 and 133mg/l of NH,-N in rumen fluid is the optimum to stimulate microbial protein synthesis and a maximum of 50mg/l is enough to stimulate microbial growth. Despite the significantly low ammonium nitrogen reported in ND, it was still within the range recommended by Satter and Slyter (1974). The high ammonium nitrogen obtained in NS could be due to the extra nitrogen added to the incubation media. The IVGP according to Lopez *et al*. (1998) and France *et al*. (2000) gives an indication of the extent to which the carbohydrate of the substrate is being fermented by anaerobic microbes from the rumen. The higher the IVGP, the higher the potential degradability of the substrate. The lack of significant difference in IVGP between the IOL in NS and ND media could suggest that the IOL has the potential to boost digestibility when fed to ruminants. It also indicates an inefficient utilization of the ammonium nitrogen in the NS media by the fiber degrading microbes. Despite the relatively high ammonium nitrogen in the

NS media which was partly due to the additional nitrogen added, it did not result in a significantly higher IVGP. The pH of the incubation media was not affected by the IOL substrate as they all fell within the optimum pH required for cellolysis and microbial protein synthesis (VanSoest, 1994; Russell *et al*., 1992).

CONCLUSION AND RECOMMENDATION

The addition of nitrogen to the incubation media did not significantly increase the in vitro gas production (IVGP) of IOL. The use of IOL as feed supplement has the potential to improve digestibility. Further study is recommended to determine the palatability and intake of IOL in ruminants.

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Mesenchymal Stem Cells in the treatment of Cerebral Ischemic Injury

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ABSTRACT

Mesenchymal stem cells (MSC) are undifferentiated adult stem cells capable of self-renewal and differentiation with a broad tissue distribution essential for tissue repairing and maintenance. These cells are isolated and expanded *in vitro* and kept as stem cells throughout many generations while maintaining its capability of differentiation when receiving appropriate stimuli. They have intrinsic multilineage potential, and as such, under special experimental conditions, are capable of differentiating into neuronal and glial cells, both *in vivo* and *in vitro*. The MSC migrate to the injured site after being intravenously injected, and in there promote endogenous cell proliferation, diminish apoptosis, and reduce the neurological deficitsresulting from cerebral ischemia. In this review we describe the many actions that the MSC exert on the injured nervous tissue, through their direct, paracrine, and systemic effects.

Keywords: Mesenchymal stem cells, cerebral ischemia, apoptosis, neuroprotection, neuroregeneration, angiogenesis, neurogenesis.

INTRODUCTION

The mesenchymal stem cells (MSC) have been found in a variety of tissues including adipose tissue, pericytes, muscles, organs and umbilical cord (Meirelles *et al*., 2008). In the bone marrow, they represent a rare population, less than 0.1% of nucleated cells. These cells have multilineage differentiating capabilities and participate reconstructing a variety of tissues (Pittenger, 1999; Argôlo Neto *et al*., 2012; Monteiro *et al*., 2012). These multipotent characteristics suggest that the MSC are responsible for repairing and maintaining all tissues in the body (Caplan, 2009).

The MSC play an important role protecting tissues, releasing growth factors, molecules and cytokines that allow local secretion of neurotrophic factors that enhances neurogenesis, and angiogenic factors that improve blood flow in the injured site through neoformation and reconstruction of the damaged vessels (Kinnaird *et al*., 2004 and Uccelli *et al*., 2011). Other roles of the MSC, through paracrine actions, include stimulation of synaptic connections and *remyelination* of damaged axons, reduction of apoptosis and regulation of inflammation (Seo e Cho, 2012). Even though these cells are not present in the ischemic site of injury, they are capable of secreting nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and increase expression of anti-inflammatory cytokines such as IFN-γ, and IL-10, which may be beneficial for repairing and rearranging the neuronal connections; induction of regeneration; stimulating neurogenesis; axonal growth; and inflammatory response and tissue protection after spinal injury (Kurozumi *et al*., 2004; Lu *et al*., 2005 and Quertainmont *et al*., 2012). This suggests that the effects of cellular therapy in ischemia are not directly related to the presence of these cells in the brain, since there is a functional recovery even when there is no evidence that MSC are present in the cerebral parenchyma thus, indicating that they are capable of acting from a distance, i.e., by systemic immunomediated mechanisms (Borlongan *et al*., 2004; Bacigaluppi *et al*., 2009 and Brenneman *et al*., 2010).

In this review we discuss the main actions of the MSC associated with repairing and protecting the CNS from ischemic injuries (Figure 1).

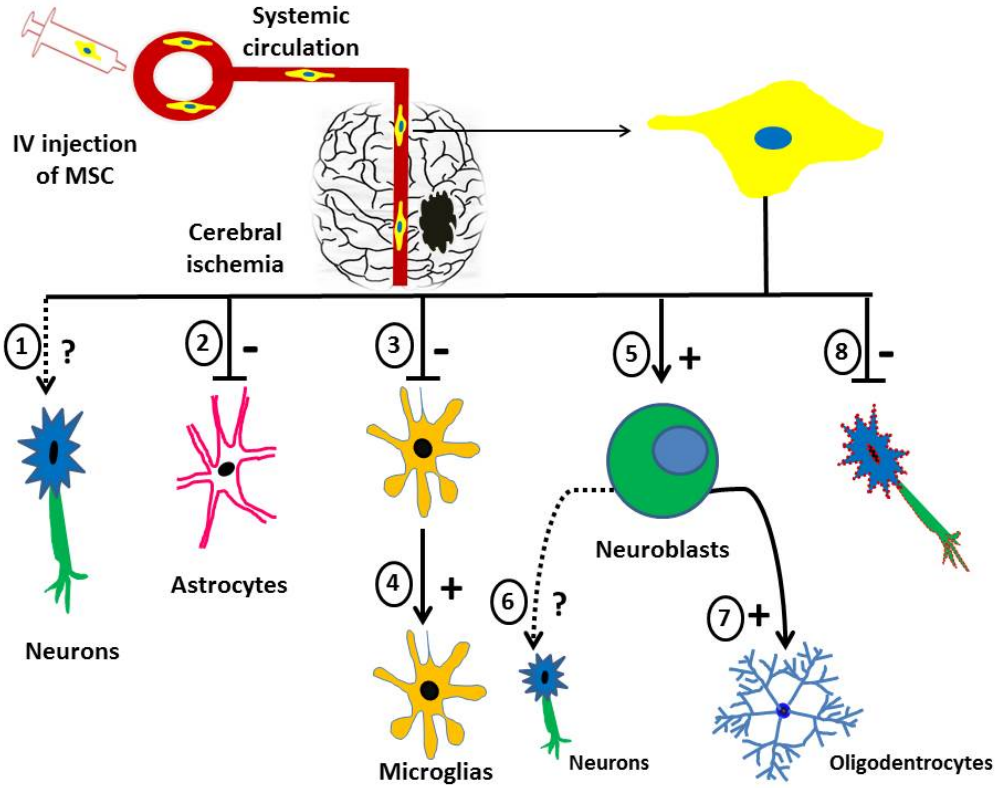

Figure1. The main neuroprotective effects of the MSC. 1- transdifferentiation, 2- astrocyte proliferation inhibition, 3- microglia proinflammatory response inhibition, 4- activation of microglial repair, 5- stimulates neuroblasts, 6- neuroblasts unknown action in neurons, 7- neuroblasts differentiation into oligodentrocytes, 8- inhibition of neuronal apoptosis. IV, intravenously, unknown mechanism; +, stimulation; - inhibition; dotted lines, indicate lack of strong evidence for the phenomenon to occur; solid line, known action. (Modified from Uccelli *et al*., 2011).

Modulation of the Inflammatory Response

Inflammation is one of the main consequences of cerebral ischemia due to the blood-brain-barrier rupture allowing for neutrophil and lymphocyte infiltration resulting in increased pro-inflammatory enzymes such as nitric oxide synthase (NOS) and proteases (Del Zoppo *et al*., 2000 and Wang *et al*., 2007). The MSC can be used for regeneration and repair therapy because they are capable to migrate to the injured site and have systemic immunomodulatory properties promoting effects on the tissue even when not present in the injured site (Bacigaluppi *et al*., 2009 and Quertainmont *et al*., 2012). They are capable to differentiate into neurons and glial cells, secrete cytokines, as well asneurotrophic and angiogenic factors that stimulate tissue repair and the migration of neuronal precursor cells (Li *et al*., 2002a and Wakabayashi *et al*., 2010).

Studies demonstrate that when MSC are transplanted into animals submitted to cerebral ischemia, these are capable to modulate the inflammatory process by reducing the buildup of Iba-1+, a microglia/macrophage-specific calcium-binding protein. Iba-1+ plays a role in the actin aggregation and participate in membrane ruffling, i.e., the formation of a motile cell surface that contains a meshwork of newly polymerized action filaments, which facilitate cellular migration and phagocytosis by the activated microglia (Ohsawa *et al*., 2004). Therefore, a reduction in Iba-1+, provided by the transplanted MSC, contribute to inhibit the pro-inflammatory expression that reaches not only the infarcted, but also the penumbra areacontributing to a reduction in ischemic expansion (SHEIKH *et al*., 2011). A significant reduction in the volume of the lesion is evident in the first few days and maybe associated with the greater production of neurotrophic factors by the MSC ensuring a neuroprotective action (Wakabayashi *et al*., 2010).

The MSC inhibit a series of pro-inflammatory molecules such as iNOS which reduces the production of NOS, cyclooxigenase-2 (Cox-2), IL-1β, IL-8, and monocytes chemoattractant protein-1 (MSP-1), which are capable of amplifying cerebral ischemia (Del Zoppo *et al*., 2000).

The activation of microglia may be modulated by neurons that inhibit inflammation (Tian *et al*., 2009). The neurons, as well as the endothelial cells diminish the microglial activation CD200dependent, a cell surface receptor that contains immunoglobulin domains, is found in the microglia and contribute to maintain themicroglia in a quiescent state (Amor *et al*., 2010). Similarly to neurons and endothelial cells, the MSC sufficiently increase the expression of CD200 in the presence of the, anti-inflammatory, cytokine IL-4, necessary to exert an anti-inflammatory effect modulating the microglia responses (Mcguckin *et al*., 2013).

Secretion of Neurotrophic Factors

The MSC favors themicroenvironmental conditions necessary to improve the region with cerebral damage by producing neurotrophic factors that protect or activate the endogenous reparation mechanisms of nervous tissue (Li *et al*., 2002). Substances that positively interfere in survival, differentiation and neuronal function of CNS are the neuronal growth factor (NGF), neutrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), hepatocyte growth factors (HGF), basic fibroblast growth factor (bFGF), and vasoendothelial growth factor (VEGF) (Uccelli *et al*., 2011). The NGF, secreted by the MSC, promotes phosphorylation, and thus, activates C reactive protein (PCR). The

phosphorylation of PCR stimulates the neuronal plasticity, acts in the regeneration capacity and in the prevention of the sympathetic neurons` death (Pierchala *et al*., 2004). Similarly, the BDNF plays a role on cell survival by promoting axonal regeneration, forming new synaptic connections (Coumans *et al*., 2001), and increasing the stimuli for the neural stem cells (NSC) differentiation, and also by protecting the neurons located in the damaged tissue (Barnabé Heider and Miller, 2003).

Comparative analysis demonstrate that bone marrow derived MSC release two folds more BDNF in relation to the adipose tissue derived MSC, and in general, cells derived from other types of tissues secret different growth factors. Therefore, the variations in neurotrophic factors from different MSC populations possess specific effects for each secreting cell types and may be chosen for a particular neurodegenerative disease (Razavi *et al*., 2013).

Studies in vivo demonstrate that MSC present Trk receptors, a family of tyrosine-kinase receptors that regulate the synaptic growth in the nervous tissue of mammals and participate in neuronal survival and differentiation. The Trk ligands are neurotrophins, a family of growth factors essential for CNS function. The NT-3, a neurotrophin that supports the neuronal survival, plays a role in the chemoattraction of the MSC due to its elevated affinity for Trk, contributing to MSC migration to the site of tissue damage (Chen *et al*., 2013).

The NSC release GDNG, which possesses high affinity for motor neurons, and warrant neuroprotection to monoaminergic and dopaminergic neurons in the nigro-striatal tract, and thus may be indicated for the treatment of degenerative diseases in this path, such as Parkinson´s disease (Whone *et al*., 2012). The GDNF´s expression significantly reduces apoptosis, providing neuroprotection to rats exposed to hypoxia (Yang *et al*., 2013), and yet increase the number of neuromuscular junctions (Suzuki *et al*., 2008).

The paracrine secretions of HGF by MSC significantly decreases demyelination due to a greater reactivity in the basic protein of myelin. The improvement of neurological recovery is attributed to the remyelination of nervous fibers and by axonal regeneration in vivo models of encephalic vascular ischemic and hemorrhagic accidents (Liu *et al*., 2010).In vitro data show that there are anti-apoptotic effects in neurons (Zhang *et al*., 2000a).

The bFGF is a polypeptide that promotes protection to the CNS cells. Its release by the MSC diminishes the infarct size in models of focal cerebral ischemia, such as in the rat. Also, it was demonstrated that intravenous administration of bFGF produces persistent reduction in the infarct volume at least up to three months after focal cerebrovascular accident (Sugimori *et al*., 2001).

The MSC transplant improves the angiogenesis after cerebral ischemia. However, it is impossible for the MSC to differentiate into endothelial cells forming new micro vessels due to the limited number of these cells. The MSC produce many growth factors, including VEGF that promotes angiogenesis in rat models of cerebral ischemia, and also significantly reduces functional deficits (Zhang *et al*., 2000b).Additionally, they play a role in the survival of nervous cells; stimulate axonal growth and the proliferation of the Schwann cells (Sondell *et al*., 1999).

Neurogenesis and Glial Activation

The subventricularzone (SVZ) in adult mammals contains neural stem cells (NSC) that differentiate and originate neuroblastsDcx+. In rats, the latter, in physiological conditions

migrate and reach the olfactory bulb. In models of encephalic ischemia, the neuroblats Dcx+, mediated by the factor derived from stromal cells 1α (SDF-1α) singnalingmigrate to the region suffering from ischemia. However, most of these neuroblasts die during their migration to the ischemic area due to apoptosis (Zhang *et al*., 2006). The MSC, implanted in models of ischemic injuries, influence the increase in NSC proliferation in the SVZ, and the survival of newly formed neuroblasts. One week after MSC infusion, there will be an increase in production of Dcx+ cells in the SVZ, thus intensifying neurogenesis. Therefore, the MSC increase the differentiation of the NSC due to secretion of growth factors favorable to neurogenesis, and promote the survival of neuroblasts that migrate to the ischemic area (Yoo *et al*., 2008).

Neurogenesis occurs in the SVZ and in the subgranularzone (SGZ) of the hippocampal dentate gyrus. However, the low survival rate of newly formed cells limits tissue repairing. In models of cerebral ischemia, the transplant of MSC increased proliferation and differentiation of NSC in the SVZ, increased the ratio of newly formed neurons, and of total cell proliferation. Histological analyses confirmthat transplanted cells had a significant survival rate at least three weeks after transplantation, and that it was possible to observe the expression of BDNF, which participates in neuronal migration (Kan *et al*., 2011). The NSC migrates in SVZ to the border zone of the ischemic area and differentiates into neurons, reduces apoptosis in rats treated with MSC improving the functional capacity after ischemia (Bao *et al*., 2011).

The astrocytes modulate the microenvironment around the neurons, ions flux, neurotransmitters, cell adhesion molecules, signaling molecules and release a great number of neuronal growth factors. In response to the ischemic event, in the second day, active astrocytes appear in the site of the lesion, and disappear five weeks later (Groves *et al*., 1993).Physiophathological studies demonstrate that only a few cells survive in the infarct area. After the third day, there is an increase in astrocytes that express glial fibrillary acidic protein (GFAP) and vimentin, mainly in the penumbra zone, and after the seventh day, these were found in the ischemic area as well (Li *et al*., 2005 and Wakabayashi *et al*., 2010). Reactive astrocytes are characterized by an intense immunoreactivity to the GFAP protein and are responsible for forming the glial scar. However, the intense presence of astrocytes in the penumbra zone inhibits the growth and regeneration of axons (Fitch E Silver, 2008).

The MSC implant, in models occluding the medial cerebral artery, demonstrated that after the third day there was a buildupreduction of GFAP+ astrocytes in the penumbra zone, and in the seventh day in the ischemic area (Sheikh *et al*., 2011). The decreasedthickness of gliosis allows axonal growth and formation of new synapses (Li *et al*., 2005).

Studies demonstrate increased expression of GAP-43 in the axons of neurons in the SVZ of rats. The Gap-43 is an essential protein for the axon and pre-synaptic region where high levels are expressed in neuronal growth cones during development and axonal regeneration (Benowitz and Routtenberg, 1997).The MSC propitiate the development of new axonal connections with intracortical (in the penumbra zone) axonal projections constituting a new network among the neurons promoting functional neurological recovery (Li *et al*., 2005).When infused after cerebral ischemia in rats, GAP-43 induces differentiation of the NSC in oligodendrocytes affording an improved functional recovery, possibly due to oligodentrogenesis stimulation (Rivera *et al*., 2006).

Angiogenesis Induction

The angiogenesis, that occurs in cerebral ischemia aid blood flow restoration improving the offer of oxygen and nutrients to the affected tissue, and thus is essential for neurological recovery (Krupinski *et al*., 1994). In patients with encephalic vascular accident (EVA), the degree of angiogenesis is correlated with survival, mainly in those with a greater microvessel density in the penumbra zone (WEI *et al*., 2012). In animal models, the angiogenesis may be amplified with MSC treatment because these cells migrate to the injured site of the nervous tissue (Li *et al*., 2002b), andrelease growth factors such as VEGF and bFGF (Chen *et al*., 2003).

Rats with induced EVA and treated with MSC had an increase in the number of VEGF-positive cells distributed throughout the ischemic cortex, detected by quantitative and immunofluorescence analysis (Guo *et al*., 2012).

After cerebral ischemia, the MSC proliferate and remodel the cortex microvasculature improving collateral blood flow, which allows identifying the presence of angiogenic factors in the penumbra zone (Whitaker *et al*., 2007 and Wei *et al*., 2012). It was demonstrated that after the transplant, in hypoxic condition, VEGF synthesis is elevated and bone marrow derived MSC are stimulated to differentiate into endothelial cells favoring angiogenesis and the performance during neurological and behavioral tests (Li *et al*., 2002b and Caplan and Dennis, 2006).

Antiapoptotic Effect

The death of neurons and glial cells are reduced by the trophic factors secreted by MSC which throughparacrine effectsincrease the survival of nervous cells in the cerebral ischemic site and reduces apoptosis (Chen *et al*., 2003 and Caplan e Dennis, 2006). The protection of the cortical neurons by the MSC in models of ischemia could be mediated by different mechanisms, such as direct MSC effects on the neurons and by secretion of factors that stimulate astrocytes to produce neuroprotective factors (Scheibe *et al*., 2012).

One of the main mechanisms that underlie the antiapoptotic effects of the MSC is the increase in NGF, BDNF, and NT-3 that activate an Akt-dependent pathway, also known as kinase protein B. The Akt protein modulates a large number of molecules that participate in cell proliferation and also inhibits apoptosis (Inoki *et al*., 2002). The MSC significantly super express Akt gene after the second day of the ischemic injury returning to basal levels in the eighth day. During this period apoptosis is inhibited (Kim *et al*., 2010).

CONCLUSION

The MSC participate in tissue protection, releasing growth factors, molecules and cytokines that allow, in the site of tissue damage, secretion of neurotrophic factors that favor neurogenesis, and angiongenic factors that improve blood flow due to neoformation and/or reconstruction of damaged vessels. Besides neuro and angiogenesis, the MSC also potentiate the formation of synaptic connections and remyelination of injured axons, reduce apoptosis and diminish inflammation. Furthermore, these cells are capable of acting from a distance modulating the action of the immune system.

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Effect of Sweet Basil (*Ocimum Basilicum*) Leaf Extract as a Spice in Hamburger

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ABSTRACT

This study was conducted at the meat processing unit of the University for Development Studies (UDS), Nyankpala campus to determine the effect of sweet basil (*Ocimum basilicum)* leaf extract as a spice in hamburger. A total of 4 kg of meat (pork) was used. Two (2) g, 4 g and 6 g basil leaves were boiled separately in 0.5 liters of water for treatment 2, treatment 3 and treatment 4, respectively for 10 minutes. Thus each product contained the following: T1) control (without basil), T2) with 10 ml of basil extract per 1 kg of meat, T3) with 10 ml of basil extract per 1 kg of meat, T4) with 10 ml of basil extract per 1 kg of meat, Sensory analyses were conducted to examine the effect of basil on the sensory characteristics of the products. Sensory characteristics were not significantly different. There were significant differences in the protein contents of the products which cannot be solely attributed to the inclusion of basil leaf extract since the trend was not consistent. There were significant differences in the moisture, fat and pH content of the products.

Keywords: hamburger, basil extract, sensory characteristics, nutritional composition.

INTRODUCTION

Meat refers to the flesh of a slaughtered animal that is eaten as food and this may include skeletal muscle, fats and other tissues (Lawrie and Ledward, 2006). Meat contains high amount of essential amino acids that play a major role in the growth and development of our bodies (Warries, 2010). In Ghana, meat is mainly from both ruminants (cattle, goats, sheep), and the non-ruminants such as pigs and poultry (domestic fowl, guinea fowl and ducks) (Adzitey, 2013). Meat processing can be defined as the procedures that involve the addition of ingredients or mechanical actions that converts fresh meat into specific products (Teye, 2007). Meat processing is done to preserve or extend the shelf life and to improve upon the flavour and tenderness of meat and meat products (FAO, 2007). Meat processing can also help to add

value to PSE and DFD meats (Adzitey, 2011; Adzitey and Nurul, 2011; Adzitey and Huda, 2012).

Hamburger is a product consisting of a cooked patty of ground meat usually placed inside sliced bread (Kenda, 1990). There have been so many claims of the invention of hamburger, but one of the earliest claims come from Charlie Nagreen, who in 1885 sold a meatball between two slices of bread at the Seymour Fair now called Outagamie County Fair (Ozersky, 2009). Originally, burgers were made from beef but in recent years chicken, pork and mutton burgers have become more common. A common feature of burgers is that it consists of minced meat blended with salt and spices, mainly black and white pepper and in some instances also herbs, garlic or onions are added. Burgers are stored frozen and individually pan-fried or grilled before consumption. Burgers are often served on bread rolls or buns with slices of cheese, mayonnaise, mustard or green salad (FAO, 2007).

Spices are esoteric food adjuncts that are used as flavouring agents and as preservatives in meat products (Srinivasan, 2005). The leaves of sweet basil plant have been among the most important spice and herbs in India and other regions in Asia, having been planted over 5,000 years (Darrah, 1980). The sweet basil plant contains anti-microbial, anti-oxidant and other medicinal properties that play a major role in the health of humans (Brandi *et al.*, 2006). It is traditionally used for supplementary treatment of stress and asthma as a spice product in India (Srinivisan, 2005). It is also used as flavouring agents in chicken soups in Ghana hence the name ‘akokobesa’ and ‘koklogbe’ in Akan and Ewe, respectively (Dokosi, 1998). Recent study has been conducted on the use of basil leaf paste which gave promising result by increasing the crude protein content in meat products (Abu, 2012). Therefore this study seeks to determine the suitability of sweet basil leave extract as a spice in hamburger.

MATERIALS AND METHODS

Location of study

The experiment was conducted at the Meat Processing Unit of the University for Development Studies (UDS), Nyankpala Campus. Chemical analysis of meat products were carried out at the Spanish laboratory of UDS, Nyankpala Campus.

Preparation of basil leaf extract

Fresh basil leaves were obtained from potted plant and were washed thoroughly in water to get rid of dirt and germs. Two (2) g, 4 g and 6 g basil leaves were boiled separately in 0.5 litres of water for treatment 2, treatment 3 and treatment 4, respectively. It was boiled for 10 minutes and the extract collected using decantation method.

Preparation of burgers

A total of 4 kg pork was obtained from the Meat Processing Unit of UDS and was thawed overnight at a temperature of 4°C. The minced meat was divided into four equal parts (1kg/treatment). Each treatment contained 15 g of curing salt (sodium nitrite), 0.5 g red paper, 1.0 g black pepper, 1.0 g white pepper, and 2.0 g mixed spices (Adobo®) with or without basil leave extract. Thus, Treatment 1 contained no basil to serve as the control, Treatment 2, Treatment 3 and Treatment 4 each contained 2 g, 4 g and 6 g of 10 ml of basil leaves extract.

Products preparation and sensory evaluation

The hamburgers were grilled in an electric oven (Turbofan, Blue seal, UK) at 100°C for 30 minutes, sliced into uniform sizes of about 2 cm, and wrapped with coded aluminium foils to keep them warm and maintain the flavour. A total of fifteen (15) panelists, were randomly selected and trained according to the British Standard Institution (BSI, 1993) guidelines for panel selection and training, to form the sensory panel for evaluation of the products. The panelist used a five-point category scale to evaluate the four treatments based on the parameters in Table 1.

Table 1: Five (5) -point scale used for the sensory evaluation

Attribute	Scale				
	1	2	3	4	5
Colour	Very Pale Red	Pale Red	Intermediate	Dark Red	Very Dark Red
Aroma	Very Offensive	Offensive	Intermediate	Pleasant	Very Pleasant
Basil Flavour	Very Weak	Weak	Intermediate	Pleasant	Very Pleasant
Flavour Liking	Dislike Very Much	Dislike	Intermediate	Like	Like Very Much
Acceptability	Dislike Very Much	Dislike	Intermediate	Like	Like Very Much

The products were presented to each of the panelists, under conditions of controlled lighting and examination so that a panelist would not be influenced by another panelist. Each panelist was provided with water and pieces of bread to serve as neutralizer between the products.

Laboratory analyses of the products

The burgers were analyzed for moisture, crude protein, pH and fat (ether extract) contents according to the methods of the International Association of Official Analytical Chemists (AOAC, 1999). Analyses were conducted in triplicates; all reagents were of analytical grade.

Data analysis

The data was analyzed using the General Linear Model (GLM) of the Analysis of Variance (ANOVA) of the Minitab Statistical Package, Version 15.

RESULTS AND DISCUSSION

The result from the sensory evaluation is presented in Table 2 and it shows no significant difference ($P > 0.05$) among the four treatments. This may be due to the level of inclusion of the basil leave extract in the four treatments. These levels were probably too low to cause any change in terms of colour, aroma, flavour liking, “akokobesa” (sweat basil) flavour and overall liking (acceptability) of the products.

Colour is a major indicator of meat quality, as the appearance of a product influences consumer acceptance. It was expected that the green colouration of basil would be transferred to the products, but that did not occurred. The insignificant difference in colour ($P > 0.05$) indicates that burgers prepared with basil leaf extract would be equally liked as the control products. This is in agreement with Abu (2012), who reported that there were no significant differences among the products in terms of colour prepared with basil leaf paste up to the inclusion level of 6 g/kg meat.

Table 2: Sensory characteristics of hamburger

Parameter	T1	T2	T3	T4	Sed	Sig.
Colour	2.5	2.8	2.6	2.2	0.395	N.S
Aroma	4	3.2	3.9	3.7	0.303	N.S
Flavour liking	2.1	3.3	2	2	0.288	N.S
Basil flavour	2.2	2.6	3.1	2.5	0.407	N.S
Acceptability	1.6	2.2	2.1	2.1	0.296	N.S

Sed = Standard error of difference. N.S = not significant

Similarly, there was no significant difference ($P > 0.05$) in the aroma of the products. Aroma refers to the smell of substances perceived by people using the nose (Adu-Adjei *et al.*, 2014). Aroma causes an attraction or repulsion of people to food substances. The insignificant difference in the aroma of the products means that burgers prepared with sweet basil leaf extract up to 10 ml/kg meat would be equally accepted as the traditional meat products on the market. This result agrees with Abu (2012), who stated that there were no significant differences among the hamburgers prepared with basil leaf paste in terms of aroma up to the inclusion level of 6 g/kg of meat.

Table 3: Nutritional qualities of hamburgers

Parameter	T1	T2	T3	T4	Sed	Sig
Moisture	53.40a	52.83a	52.72a	49.78b	1.693	*
Fat	56.87b	49.32c	27.25d	58.07a	0.13	**
Protein	15.14b	16.66a	14.65c	14.31d	0.143	**
pH	6.09a	6.07b	6.05c	6.07b	0.009	**

Sed = Standard error of difference. Sig = significance. Means on the same row with the same superscript are not significantly different. * = $P < 0.05$. ** = $P < 0.01$

Table 3 shows the nutritional qualities of the burgers. There were significant differences ($P < 0.05$) in the moisture, fat, protein and pH of the products. Moisture refers to the level of water contained in products. Juiciness of a meat is greatly influenced by the moisture present. High moisture makes meat more juicy and less moisture makes meat less juicy. The storability of meat is also influenced by the amount of moisture present. Since the moisture content of T4 was significantly lower it could store better than the rest of the products. More so, T2 and T3 suggests that the microbial quality of the test products would not be affected by the inclusion of basil leaf extract up to 10 ml of 6 g of basil leaves/kg. This result agrees with Abu (2012), who reported that there were no significant differences in moisture of the hamburgers prepared with sweet basil leaf paste up to the inclusion level of 6 g/kg of meat.

According to FAO (2007), fats are added to processed meat products to make it softer and also for taste and flavour enhancement. The results obtained from the study showed that there were significant differences in the fat content of the products ($P < 0.05$). However, there was drastic reduction in treatment 3 which could be due to sampling error, since this result disagrees with findings of Abu (2012), who reported insignificant differences in fat content of hamburgers prepared with basil leaves paste included up to 6 g/kg meat.

There were significant differences in the protein contents of the products which cannot be solely attributed to the inclusion of basil leaf extract since the trend was not consistent. This results again disagrees with the findings of Abu (2012), who reported that there were consistent increased in protein content of the product as the inclusion level of basil leaf paste increases.

pH is a measure of the acidity or alkalinity of water containing substances. This result recorded a significant difference in pH of the control and test products. The control product

was significantly higher ($P < 0.01$) than the test products. FAO (2007) indicated that the pH of meat products are important for their storage, the lower the pH, the lesser favorable condition for microbial growth and therefore storability is enhanced for longer time. It can be suggested that that the test products will store better than the control product.

CONCLUSIONS

This study showed that the addition of sweet basil leaf extract (10 ml/kg) to hamburger up to level of 6 g/kg in meat had no effect on appearance of the products. Sweet basil has no effect on the flavour liking, aroma and overall acceptability of the products. However, the moisture, fat, protein and pH were significantly affected.

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Effect of Nutrition and Castration on carcass Measurements, Wholesale Cuts and Carcass Composition of Male Desert Goats

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ABSTRACT

The experiment was conducted in desert goats to evaluate the effect of nutrition and castration on carcass measurements, wholesale cuts and carcass composition of male desert goats. Seventy two male desert goats aged 4-5 months and weighing 12.1Kg (11.7-12.4 kg) were used in this experiment. The goats were divided randomly into three groups; each group (24 goats) was subdivided into two sub groups (12 goats) in Factorial experimental randomized design. The results revealed that nutrition and castration had no significant effect ($p>0.05$) on carcass length. Internal chest length, external chest length, distal foreleg length, proximal foreleg length, proximal hind leg length, foreleg circumference, hind leg circumference and eye muscle area were significantly affected ($p<0.05$) by nutrition, On the other hand, castration had no significant effects ($p>0.05$) on these measurements. Forequarter, rack, breast percentages were significantly affected ($p<0.01$) by nutrition, on the other hand, castration had no significant effect ($p>0.05$) on these cuts except loin cut percentage. Carcass dissected revealed that nutrition had greater muscle, bone and fat compared with grazing group. On the other hand, castration had no significant effect ($p>0.05$) on carcass composition except muscle to fat ratio which was significantly affected ($p<0.05$) by castration and interaction of nutrition and castration.

Keywords: Desert goats, nutrition, castration, carcass, meat.

INTRODUCTION

Sudan desert goats are found in arid and semi arid areas of Sudan, especially in Kordofan and Darfur regions and are adapted to survive under adverse conditions of feed limitations and water scarcity (Mason and Mule, 1960). Total annual red meat production in Sudan is estimated at 8830 tones, with goats contributing about 310 tones and annual live goat exports to the Arab world exceed 16.500 head (MAR, 2003). Goats have been a common source of meat in many tropical and developing countries and they are more important meat producing

animals compared to sheep (Mahgoub and Lodge, 1998). Hango *et al.* (2007) reported that carcass weight and dressing percentage increased significantly ($p<0.05$) with increasing amount of concentrate. Castration of animals is a common management practice that imposes unnecessary pain and stress and may reduce performance (Hopkins-Shoemaker *et al.*, 2004). The presence of testicular hormones is related to greater muscle growth capacity in intact males (Arnold *et al.*, 1997). Castration in goats has an advantage of eliminating the strong male odor present in bucks. Un-castrated and sexually mature goats are difficult to sell or they may have low market price because of their strong male taint. Castrations also affect growth and carcass composition (Solomon *et al.*, 1991). Generally Castration reduced slaughter weight and carcass weight and improved the juiciness. The objective of this research is to study the effect of nutrition and castration on carcass measurements, wholesale cuts and carcass composition of male desert goats.

MATERIALS AND METHODS

Experimental animal's management

This work was conducted at El-Obeid Agricultural Research Station, North Kordofan State, Sudan .Seventy two male desert goats aged 4-5 months and weighing 12.1Kg ranged between (11.7-12.4 kg) were used in this study. The goats were divided randomly into three groups, each group (24 goats) was subdivided into two sub groups (12 goats); one sub group was castrated while the other was left intact. Group one was kept on grazing only which consist of dry grasses (Bano, *Eragrotis turmula*, Sheeleni, *Zornia glochidiata*, Haskaneet, *Cenphrus spp)* and browse (Sedeer leaves, *Ziziphus spina chritis*, Hegleeg leaves, *Balanite aegyptiaca* and Ushar dried leaves and flower, *Calotropis purocera*). Group two was kept on grazing plus concentrate supplement with experimental diet which consist of (wheat bran 38%, Dura grain fetareta 30%, ground nut cake 20%, ground nut hay 10%, sodium chloride 1%, and limestone 1%) given at a rate of 324g/head/day. Group three was *ad libitum* fed the experimental diet (zero grazing). The chemical composition of range plants and experimental diet were shown in Table (1). Live weight of each animal was recorded at the start of experiment, then weekly until the end of trial (90 days), using spring balance. The animals were weighed in the morning (before grazing), following over night fast except for water.

Table 1. Chemical composition of range plants and experimental diet

Chemical composition	Range of plant	Experimental diet
Dry matter (DM %)	95.9	98.6
Organic matter (OM%)	84.2	87.7
Crude protein (CP%)	10.0	22.0
Crude fiber (CF%)	32.0	25.0
Ether extract (EE%)	7.0	12.0
Nitrogen free extract (NFE%)	36.0	31.0
Ash (%)	10.0	08.0
Energy density(MJME/Kg DM)	9.0	12.0

*Calculate as in MAFF (1972)

Slaughter procedure and data collection

At the end of the experiment which extended for 90 days twelve animals from each treatment group were slaughtered at the end of the experiment. Slaughter was performed according to Muslim practice by severing jugular vessels, esophagus and trachea without stunning. Following skinning and evisceration the external and internal offals including gut content were weighed. The hot carcass weight was recorded; the carcass was split along midline.

The weights of the carcass halves were recorded. On the left half carcass measurements were done according to procedure of Owen (1975) then the left half was cut into wholesale cuts, each cut was dissected into lean, fat and bone and then tissues separately were weighed, dissection was carried out according to procedure outlined by Cuthberton *et al.* (1972).

Statistical analysis

The data were statistically analyzed according to factorial experimental randomized design as a 3×2 (management Vs sex). Multiple range tests were used to compare the means.

RESULTS AND DISCUSSION

Effect of nutrition and castration on carcass measurements (cm) of male desert goat:

The effect of nutrition and castration on carcass measurements is displayed in (Table 2). The results showed that the internal and external chest length and Eye muscle area were significantly higher (P<0.001) where Distal foreleg length, Proximal foreleg length, Distal hind leg length, Proximal hind leg length, Foreleg circumference and Hind circumference were significantly high (P<0.05). Effect of castration on carcass measurement did not show any significant effect. Most of the carcass measurements increased with supplementation of grazing and with *ad libitum* feeding of concentrates which might be due to the improvement of the nutrition status of the animal resulting in increased tissue growth. Castration had no significant effect on some carcass measurements because castration spared energy which was utilized for tissue growth.

Table: 2.Effect of nutrition and castration on carcass measurements (cm) of male desert goat

Parameters	Nutrition(N)					Castration (C)							
	Grazing	Grazing + supple	Zero grazing	SE	LS	Grazing		Grazing+ supple		Zero grazing		SE	LS
						Entire	Castrate	Entire	Castrate	Entire	Castrate		
No. of Animal	24	24	24	-	-	12	12	12	12	12	12	-	-
Exp. period (days)	90	90	90	-	-	90	90	90	90	90	90	-	-
Carcass length	38.3	40.9	42.5	1.3	NS	37.5	39.0	41.4	40.4	43.7	41.2	1.1	NS
Internal chest length	19.0[a]	19.7[b]	21.3[b]	0.4	* **	18.2	19.8	19.4	19.9	21.3	21.3	0.3	NS
External chest length	21.5[a]	23.6[b]	24.8[b]	0.5	* **	20.4	22.5	23.5	23.7	25.1	24.5	0.4	NS
Distal foreleg length	17.8[a]	18.6[b]	19.9[b]	0.3	* *	17.2	18.4	18.3	18.9	20.4	19.3	0.3	NS
Proximal foreleg length	14.1[a]	14.4[b]	15.0[b]	0.2	*	13.6	14.6	14.4	14.3	15.3	14.6	0.2	NS
Distal hind leg length	25.4	25.7	26.5	NS	* *	25.0	25.7	24.4	26.8	26.1	26.8	0.4	*
Proximal hind leg length	14.4[a]	16.4[b]	16.1[b]	0.6	*	13.9	14.8	17.4	15.3	15.4	16.8	0.8	NS
Foreleg circumference	16.7[a]	18.6[b]	18.7[b]	0.5	**	17.0	16.3	18.8	18.3	19.0	18.3	0.4	NS
Hind circumference	32.2[a]	36.0[b]	36.3[b]	1.1	**	34.2	30.1	38.3	34.2	37.5	35.1	0.9	NS
Eye muscle area	3.2[a]	4.5[b]	5.8[b]	0.4	***	2.9	3.6	4.7	2.9	2.9	2.9	0.3	NS

[ab] Values in same rows with different superscripts differ at P<0.001, P<0.01 and P<0.05 respectively, NS= non significant

Effect of nutrition and castration on primal cuts (%) of male desert goat

Wholesale cuts proportions of goat increased with concentrate supplementation and in the *ad libitum* feeding of concentrates and that of forequarter rack and breast cuts increased significantly (Table 3). This result was in contrast with Srivastva and Sharma (1997) who observed that none of the cuts (leg, loin, rack, shoulder, breast and shank) were significantly affected by dietary treatment in Jumunapari goats. Castration had no significant effect on percentages of forequarter, rack, breast and leg cuts, but the forequarter and breast were heavier in entire than in castrates (Table 3). These findings agreed with the findings of Simela *et al.* (2011**)** who reported no significant differences between entire and castrated male desert

goats in the percentage of carcass wholesale cuts. This trend was also similar to the findings of Robles *et al.* (1985) which revealed that the major carcass cuts of goat as the forequarter and the breast were heavier for entire than for castrates. Male sex hormones in entire individuals might be responsible for the increased weight of forequarter and breast. Growth is differential and carcass tissues have different growth rates that are mainly regulated by sex hormones (Devendra and Burns, 1983).

Table 3. Effect of nutrition and castration on primal cuts (%) of male desert goat

Parameters	Nutrition(N)					Castration (C)							
	Grazing	Grazing+ supple	Zero grazing	SE	LS	Grazing		Grazing+ supple		Zero grazing		SE	LS
						Entire	Castrate	Entire	Castrate	Entire	Castrate		
No. of Anim	24	24	24	-	-	12	12	12	12	12	12	-	-
Exp. period (days)	90	90	90	-	-	90	90	90	90	90	90	-	-
Forequarter	42.8[a]	49.7[b]	48.2[b]	1.5	**	49.0	47.4	50.6	48.7	42.8	42.7	1.2	NS
Leg	32.8[a]	37.4[b]	37.2[b]	1.9	NS	37.3	37.0	37.5	37.3	31.9	33.6	1.5	NS
Rack	8.1[a]	11.4[b]	11.9[b]	0.8	* *	13.7	10.1	11.8	11.0	7.8	4.8	0.7	NS
Breast	5.4[a]	9.5[b]	9.7[b]	0.6	* **	10.3	9.0	9.6	9.3	6.1	4.6	0.5	NS
Loin	9.3	9.5	9.6	0.7	NS	11.1[a]	8.1[b]	10.2[a]	8.8[b]	9.1[a]	9.5[b]	0.5	*

[ab] Values in same rows with different superscripts differ at P<0.001, P<0.01 and P<0.05 respectively, NS= non significant

Effect of nutrition and castration on carcass composition of male desert goat

The data in Table (4) revealed that total muscle of zero grazing goats group and grazing + supplementary group had recorded greater percentage compared with grazing group, and this agreed with the finding of Elkhidir (1989) and Hassaballa (1996) who reported that the total muscle percentage was 64. On the other hand castration had no significant effect on total muscle percentage (Table 4), but entire goats had relatively more muscles compared with castrates, which was in line with findings of Smith (1982) and Devendra and Burns (1983) who reported that intact goat males had less fat and more muscle and bone than castrates and females. Wilson (1958) and Koyuncu *et al.* (2003) reported that castration did not affect tissue distribution in the carcass except intramuscular fat which is known to be a more variable tissue in quantity and distribution.

Table 4. Effect of nutrition and castration on carcass composition of male desert goat

Parameters	Nutrition(N)					Castration (C)							
	Grazing	Grazing + supple	Zero grazing	SE	LS	Grazing		Grazing+ supple		Zero grazing		SE	LS
						Entire	Castrate	Entire	Castrate	Entire	Castrate		
No. of Anim	24	24	24	-	-	12	12	12	12	12	12	-	-
Exp. period (days)	90	90	90	-	-	90	90	90	90	90	90	-	-
Total muscle (g)	1238.8[a]	1901.0[b]	2249.0[b]	0.5	***	1323.0	1154.0	1944.5	1858.0	2194.0	2304.1	102.5	NS
Muscle (%)	58.5[a]	66.7[b]	66.0[b]	2.2	*	59.9	58.1	77.0	62.3	63.2	68.8	1.8	NS
Total bone weight (g)	625.0a	779.3[b]	853.6[b]	29.8	* **	676.0	573.9	800.5	758.0	900.0	806.8	24.4	NS
Bone (%)	30.3[a]	28.4[b]	26.1[b]	0.9	* **	31.4	29.2	31.1	25.7	25.5	26.7	0.7	NS
Total fat weight (g)	53.2[a]	123.1[b]	338.0[b]	38.8	* **	38.0	70.0	97.0	149.1	310.0	365.9	31.7	NS
Fat (%)	2.8[a]	4.0[b]	4.6[b]	0.9	* **	1.6	3.4	3.2	4.8	4.0	5.1	0.7	NS
Muscle: fat	23.3[a]	15.4[b]	14.3[b]	14.0	*	21.5[a]	24.5[b]	13.0[a]	17.0[b]	14.1[a]	13.5[b]	11.7	*
Muscle: bone	2.0[a]	2.5[b]	2.6[b]	0.1	***	2.0	2.0	2.4	2.0	2.4	2.8	0.1	NS
(Muscle+ fat): bone	2.0[a]	2.6[b]	3.0[b]	0.2	***	2.0	2.0	2.6	2.6	3.0	3.0	0.1	NS

[ab] Values in same rows with different superscripts differ at P<0.001, P<0.01 and P<0.05 respectively, NS= non significant

CONCLUSIONS

It could be concluded that during management practices of goat that involved grazing, concentrate supplementation of grazing, zero grazing and castration. Zero grazing group recorded highest carcass measurements on the other hands, grazing plus supplementation

group registered the highest values in forequarter and leg cuts, muscles percentages and muscles: fat.

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Review Article

Live Body Weight Estimation in Small Ruminants-A Review

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ABSTRACT

Proper measurement of live body weight, which often is hard in the village settings due to lack of weighing scales, is a prerequisite for achieving so many lofty goals that are always associated with either medical or economic status of the animals. Under standard conditions, properly calibrated livestock scales are the most accurate and consistent method for determining body weight. Under farm conditions however, where scales and records may be absent, it may be difficult to know the weight of sheep and goats. Procedures for estimating weight of small ruminants in such conditions include the use of weight band, visual appraisal, and use of body linear measurements among others. All these measurements give estimates of the animals' live body weights however, it has been shown in many studies that the heart girth is the most appropriate and confident parameter in live weight estimations for sheep and goats.

Keywords: Estimation, Body Measurements, Lives Weight, Small Ruminants.

INTRODUCTION

Proper measurement of live body weight, which often is hard in the village settings due to lack of weighing scales, is a prerequisite for achieving so many lofty goals that are always associated with either medical or economic status of the animals. Knowing the live bodyweight of small ruminants is important for a number of reasons, such as for breeding, correct feeding and health (Slippers *et al*., 2000). Apart from taking live weight of meat animals, researchers also use other parameters such as body length, width of pelvis, height at withers and chest girths in order to adequately evaluate live animals (Atta *et al*., 2004). Under standard conditions properly calibrated livestock scales are the most accurate and consistent method for determining body weight. Under farm conditions however, where scales and records may be absent, it may be difficult to know the weight of sheep and goats (Abegaz and

Awgichew, 2009). Some of these standard weighing scales coupled with their shortcomings are too expensive for most of small farmers (Mahieu, 2011). This has forced many farmers to rely on estimates of body weights using certain number of body characteristics which can be measured readily (Alade *et al*., 2008). Among these, body measurements have been used to predict body weight in large domestic animals (Morison, 1949; Quin, 1980; Getenby, 1991; Thys and Harduoin, 1991; Mayaka *et al*., 1995; Mayeni and Slippers, 1997). For small ruminants studies in particular, sheep (Prasad *et al.*, 1990; Aziz and Sharaby, 1993; Enevoldsen and Kristensen, 1997; Valdez *et al*., 1997; Atta and El Khidir, 2004; Riva *et al*., 2004; Afolayan *et al*., 2006; Nayak *et al*., 2008; Otoikhian *et al*., 2008; Sowande and Sobola, 2008; Edea*et al.*, 2009; Getachew *et al*., 2009; Kunene *et al*., 2009; Cam *et al*., 2010a; Iqbal, 2010; Tadesse and Gebremariam, 2010; Oke and Ogbonnaya, 2011;Mohammad *et al*., 2012; Musa *et al*., 2012; Ravimurugan*et al*., 2013; Shirzeyli *et al*., 2013; Younas *et al*., 2013) and goat (Tandon, 1965; Mukherjee *et al*., 1981; Mohammed and Amin, 1997; Das *et al*., 1990; Prasad *et al*., 1990; Hassan and Ciroma, 1991; Ulaganathan *et al*.,1992; Slippers *et al*., 2000; Nsoso *et al.*, 2004; Singh and Mishra, 2004; Gül *et al*., 2005; Adeyinka and Mohammed, 2006; Khan *et al*., 2006; Moaeen-ud-Din *et al*., 2006; Rahman, 2007; Fajemilehin and Salako 2008; Pesmen and Yardimci, 2008; Cam *et al.*, 2010b; Tsegaye, 2013).
This review paper would therefore highlight the different methods used in estimating live body weights of small ruminants and also serves as a weight-taking guide to village farmers, extension agents, researchers as well as the small ruminant clinicians.

Procedures for estimating weight of small ruminants

- **Weight band:** A weight band is a specially marked tape used to measure the heart girth and convert that measurement to a fairly accurate estimate of the goat's live weight. De Villiers *et al*. (2010) described this procedure. Briefly, the weight band is wrapped directly behind the shoulder blade, down the fore-ribs, under the body behind the elbow and all the way around to the point behind the shoulder blade. The ends of the weight band are overlapped on top, on the goat's spine. Lastly, the resultant weight measurement is read off the weight band in kilograms.

- **Visual appraisal**: This skill is developed through practice by estimating the weight of numerous animals without a board or weigh band. Visual determination of the weight of animals is often faced by errors like using the same estimate for more than one breed of a particular species (Otoikhian, 2008). Body structure can be deceptive when estimating weight (Slippers *et al.*, 2000). For instance, Red Sokoto goats appear lighter than they actually are because of their light bones. Apart from bones and body structure problem in estimating weight, a white animal always looks bigger than it is (Otoikhian, 2008).

- **Body Linear Measurements**: There are a number of linear dimensions which can be used to quantify the size of an animal and to estimate weight. The most widely used body linear measurements include height at withers, heart girth, chest depth, body length, fore cannon bone, rump height, distance between eyes, ear length, ear width, paunch girth and tail length. Heart girth and cannon bone length are least affected by the posture of the animal. Abegaz and Awgichew (2009) described the linear measurement as follows:

- **Height at Withers (HAW)**. This measures the distance from the surface of a platform on which the animal stands to the withers. The measurement is best made with a special measuring stick made with two arms one which is held vertical and the other at right angles to it sliding firmly up and down to record height. The sheep or goat should stand squarely on all four legs. The legs should be equally spaced, and carry equal portions of its weight.

The vertical arm of the measuring device is placed on the ground and ensures it is at a right angle to the platform. Then the other shorter arm is slide down until it just touches the shoulder at the desired point. The vertical measuring device is withdrawn and the distance is measured with a measuring tape. Alternatively, the vertical arm could have the measuring scale inscribed onto it and height read directly. This method can be used alone or in combination with the other linear measurements to get more accurate results.

- **Heart Girth (HG) or Chest circumference**: Heart girth is a circumferential measure taken around the chest just behind the front legs and withers. The measurement should be taken to the nearest 0.5 cm. HG is a highly repeatable measure though it does vary somewhat with extremes of posture and perhaps as the animal breaths. It is the basis of the many weight tapes that are available for estimating animal weight as there is a good correlation between chest circumference and body weight, within breeds, sexes, and ages of stock. More reliable HG-live body weight relationships are obtained from mature animals. In excessively hairy small ruminants, make sure to compress the hair while measuring HG.

- **Body Length (BDL)**: Body length refers to the distance from the base of the ear to the base of the tail (where it joins the body). It can also be measured as the distance from base of tail to the base of the neck (first thoracic vertebrae), or to front of the chest or to tip of the nose. Extreme care is needed to ensure that the backbone is straight in both vertical and horizontal planes.

- **Hip Width (Pin Bone Width) (HW):** Hip width is the distance between the outer edges of the major hip bones on the right and left side. The hipbones are easily located and the distance between them easily measured with a pair of large, half round or oval shaped callipers.

- **Rump Height (RH)**: Rump height is the distance from the surface of a platform to the rump using a measuring stick as described for height at withers.

- **Fore Cannon Bone Length (CB):** This is the length of the lower part of the leg extending from the hock to the fetlock in hoofed mammals. It is a well-established fact that linear development of different bones in the body is strongly related. The different parts grow in proportion to one another. It should be possible to estimate the length of a bone which is difficult to measure indirectly through its correlation with a more accessible one. The fore cannon bone is the one most commonly used. To measure fore cannon length, have the sheep or goat either stand or be held sitting on its rump. Take the front leg and bend back the hoof at the pastern and the leg itself at the knee. Use a suitable pair of large callipers, or a ruler or a measuring tape to measure the length of the main lower leg bone. For greatest accuracy, standardize your measurements using the same bony protuberances in each animal.

- **Chest Depth (CD).** Chest depth measures the distance from the backbone at the shoulder (standardize on one of the vertical processes of the thoracic vertebrae) to the brisket between the front legs.

Prediction Models

Mathematical equations (Prediction models) can be developed based on large number of actual weight-linear measurement data discussed above. The equations change the linear

measurements into weight estimates, usually via a constructed table. Individual equations can be derived based on condition, sex and age of the animal.

How are the models generated?

After the body measurements, the data could be grouped on the basis of sex, age (which according to Mitchell (1982), is determined by counting the number of permanent incisors) and breeds. Then, depending on the design, different statistical methods could be used to analyzed the relationship between the live body weight and the body linear measurements. In most of the literature however, the relationships between the body weight and linear measurements, and among the linear measurements themselves are determined by the use of Pearson's Correlation Coefficients. The body weight would then be regressed on body linear measurements using general linear model and regression analysis to generate prediction models. To determine the best fitted regression model, coefficient multiple determination (R^2), residual mean square(MS_E), error standard deviation(SD_E) and range observed in the predicted weights could be used to evaluate and compare different regression models generated(Snedecor and Cochran,1989). Also, more than one linear measurement may be used in an equation to improve predictive ability as seen in the work of Pesmen and Yardimci (2008).

Many studies in literature have used one or more of the aforementioned statistical procedures in small ruminants to generate prediction models for estimating live body weight using body linear measurements. For instance, in sheep (Afolayan*et al*., 2006; Otoikhian, 2008; Ravimurugan *et al*.,2013; Tadesse and Gebremariam, 2010; Musa *et al*., 2012; Shirzeyli *et al*., 2013; Younas *et al*., 2013) and in goats (Atta *et al*., 2004; Moaeen-ud-Din *et al*., 2006; Alade *et al*., 2008 ; Pesmen and Yardimci, 2008; De Villiers *et al*., 2009; Mahieu *et al*., 2011; Tsegaye, 2013).

Also, though very few, the use of Regression Tree Method was used instead of Multiple Regression Analysis(MRA) in analysing multiple relationships among traits, in order to predict body weight using these body measurements (Eyduran *et al.,* 2008; Mendes and Akkartal, 2009; Topal *et al.,* 2010; Muhammad *et al.,* 2012). This is because; MRA produces biased estimates under some conditions, especially the multi-collinearity problem. (Keskin *et al*., 2007a, Keskin *et al*., 2007b; Eyduran *et al*., 2009; Eyduran *et al*., 2010).

Precautions while taking body linear measurements

Since the animal body movement and body posture can introduce errors into measurements and estimated weights, Abegaz and Awgichew (2009) suggested the following precautions to be taken in order to counteract these effects:

i. When possible, choose measurements that are little affected by the animal's posture
ii. Standardize the position of all animals that are to be compared
iii. Be patient and wait for an animal to stand correctly.

CONCLUSION

Although all the linear measurements discussed above give an estimate of the animals live body weight, yet it has been shown in many studies (Yarkin *et al*., 1961; Tuncel, 1982; Valdez *et al*., 1982; Hassan and Ciroma, 1990; Koyuncu and Tuncel, 1992; Ozturk *et al*., 1994; Mohammed *et al*., 1996; Atta and El Khidir, 2004; Pesman and Yardimci, 2008) that the heart girth is the most appropriate and confident parameter in live weight estimations for sheep and goats.

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Review Article

Potential Use of *Moringa Olifera* in Poultry Diets

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ABSTRACT

This paper reviewed researches on the use of *Moringa oleifera*in poultry diets. As the price of compound feed continues to escalate due to the high expense of conventional protein sources such as fishmeal and soybean meal there is an urgent need to look for alternative sources of protein and Moringa is one of such protein sources. Moringa has excellent nutritive value and therapeutic properties. The crude protein (CP) content of Moringa ranges from 71.2 to 391.7 g/kg and varies across the plant parts with the seeds having the highest CP content followed by flowers, leaves, whole plant, stems and pods. However, Moringa contains anti-nutritional factors such as tannins, phytates, trypsin inhibitors, saponins, oxalates and cyanide, which affect protein and mineral metabolism and availability to the animal.The availability of phosphorus to the birds can be enhanced through addition of phytase to break down phytate that binds phosphorus. It is apparent from the previous studies that inclusion of Moringa in poultry diets improves performance of chickens in terms of growth rate and egg production. As the cost of Moringa can be prohibitively high in some countries, economically inclusion levels should be determined.

Keywords: Anti-nutritional factors, broilers, layers, *Moringa oleifera*, protein source.

INTRODUCTION

Livestock feed costs in developing countries are a continuing challenge. The high and increasing prices for animal feeds have compelled researchers in developing countries to direct their attention to non-conventional feeds, with particular emphasis on protein substitutes (Gaia, 2005). *Moringa oleifera* Lam is one of the 13 species of Moringaceae, which is native to India, Red Sea and parts of Africa including Madagascar. Of these, *M. oleifera* is the most widely known (Price, 2007). *Moringa oleifera* is among plants that can be integrated with livestock production to increase feed quality and availability as it can be used

as a cheap protein supplement to improve digestibility of other diets. All plant parts can be used to feed livestock.

Moringa grows best in the hot, semi-arid tropics; hence it is drought-tolerant and grows at a rainfall of 250-1500 mm per year (Martin, 2007). In its natural habitat Moringa grows up to 1 400 m of altitude, along the biggest rivers on alluvial, sandy or gravel soils (Pérez, 2005). Moringa tree prefers well-drained sandy or loam soil. It also grows on a soil pH ranging between 4.5 and 8, except on heavy clays, and it prefers neutral or slightly acidic soils. A good temperature range for the tree is 25-35 ºC though it can tolerate up to 48 ºC for limited amount of time (Pérez, 2005). The tree has an average height of 5 to 12 m (Paguia *et al*., 2012). The trees cultivated for forage are pruned to restrict the development of the crown and promote the growth of new branches (Pérez, 2005).In Botswana, Moringa tree is found in Gaborone, Mahalapye, Palapye, Francistown, Maun and other parts of the country but only a few people know the benefits and uses of this miracle tree (Nduwayezu, 2006).

The poultry industry in the developing countries is facing some challenges, one of which is an increase in the cost of feed because of high prices of protein and energy sources (Abbas, 2013). Although rich in nutrients such as protein and minerals, *Moringa oleifera* is one of those plants that have not been studied for many years but now is being investigated for its fast growth, higher nutritional value, and utilization as a livestock fodder crop (Nouman *et al*., 2013). Therefore, this manuscript endeavours to present a detailed discussion on the use of *M. oleifera* in poultry diets.

Nutrient composition of *Moringa oleifera*

Moringa oleifera tree contains high crude protein (CP) in the leaves (251 g/kg DM) and negligible content of tannins and other anti-nutritive compounds and offers an alternative source of protein to ruminants (Nouala *et al*., 2006) and non-ruminants. The nutrient composition and digestibility of morphological parts of *M. oleifera* is shown in Table 1. According to Table 1, the seeds contain high amount of CP, followed by flowers and leaves, suggesting that *M. oleifera* can be used as a protein source for both livestock and humans. The fact that the seeds contain higher CP content than other parts suggests that suggesting that they can be used as a valuable source of protein.Ojukwu (2012) stated that Moringa leaves are periodically harvested to make a sauce, locally known as "*mboum*" or can be used to feed poultry, pigs and cattle. In a recent study, Aye and Adegun (2013) in Nigeria found lower percentages of DM of MOLM to be 93.63±0.01, ash (7.96±0.03), CP (22.23±0.25), CF (6.77±0.01), EE (6.41±0.01), NFE (40.28+0.25) while gross energy was (14.790) (MJ/kg). These results indicate that nutrient composition of MOLM differs according to location and possibly stage of harvesting of Moringa leaves.

Table 1: Nutrient composition and digestibility of morphological parts of *Moringa oleifera*

Plant parts	DM	Ash	CP	EE	CF	Digestibility
Seeds, g/kg	950.0	34.8	391.7	388.0	48.0	-
Flowers, g/kg	892.5	112.1	314.8	68.0	170.0	-
Pods, g/kg	940.0	97.1	71.2	20.0	490	430.7
Leaves, g/kg	930.0	138.9	267.9	64.0	210.0	790.5
Stems, g/kg	940.0	101.1	112.3	32.0	430.0	521.7
Whole plant, g/kg	914.0	123.7	200.0	24.0	270.0	760.9

Source: Mabruk *et al.* (2010)

Morphological parts of *M. oleifera* such as leaves, stems, whole plants and pods according to their moderate to high CP content (71.2-267.9 g/kg DM) and high crude fibre content (CF) of 210.0 - 490.0 g/kg DM are considered good sources of roughage for feeding domestic ruminants for maintenance and production (Mabruk *et al*., 2010).

Multiple uses of *Moringa oleifera*

The multiple uses of *M. oleifera* are illustrated in Figure 1. It is clear from Figure 1 that there is a lot of potential for Moringa to be used as an ingredient in livestock diets. Despite the high CP content of MOLM, there are few reports in literature on feeding trials with livestock. Sarwatt *et al*. (2002) stated that both large and small-scale farmers in Tanzania grow *M. oleifera* for extraction of seed oil and thus there is potential to use the foliage for feeding livestock and the cake as a protein source. Nouala *et al*. (2006) investigated the influence of *M. oleifera* leaves as a substitute to conventional concentrate on the *in vitro* gas production and digestibility of groundnut hay and reported that *M. oleifera* leaves appeared to be an alternative source of protein for ruminant production in West African settings and can be used as supplement to diets based on crop residues/poor roughage. In combination with concentrate, *M. oleifera* leaves further improved the efficiency of concentrate utilization. Ogbe and John (2012) harvested the leaves of *M. oleifera* from Lafia in Nasarawa State of Nigeria during the rainy season in June 2011 and determined their proximate, mineral and phytochemical analysis. The proximate analysis revealed the presence of high CP (17.01% ±0.1) and carbohydrate (63.11% ±0.09), CF (7.09% ±0.11), ash (7.93% ± 0.12), ether extract (EE) (2.11% ±0.11) and fatty acid (1.69% ±0.09). The phytochemical analysis and anti-nutrients showed the presence of tannins (21.19% ±0.25), phytates (2.57% ±0.13), trypsin inhibitors (3.0% ±0.04), saponins (1.60% ±0.05), oxalates (0.45% ±0.01) and cyanide (0.1% ±0.01). The presence of these essential nutrients and minerals implies that *M. oleifera* leaves could be utilized as a source of feed supplement to improve growth performance and health status of poultry. However, the high protein content of Moringa leaves must be balanced with other energy feeds. Martin (2007) suggested that cattle feed consisting of 40-50% Moringa leaves should be mixed with molasses, sugar cane, young elephant grass, young sweet sorghum plants, or whatever else is locally available. Several researches have demonstrated that inclusion of *M. oleifera* in livestock diets has beneficial effects.

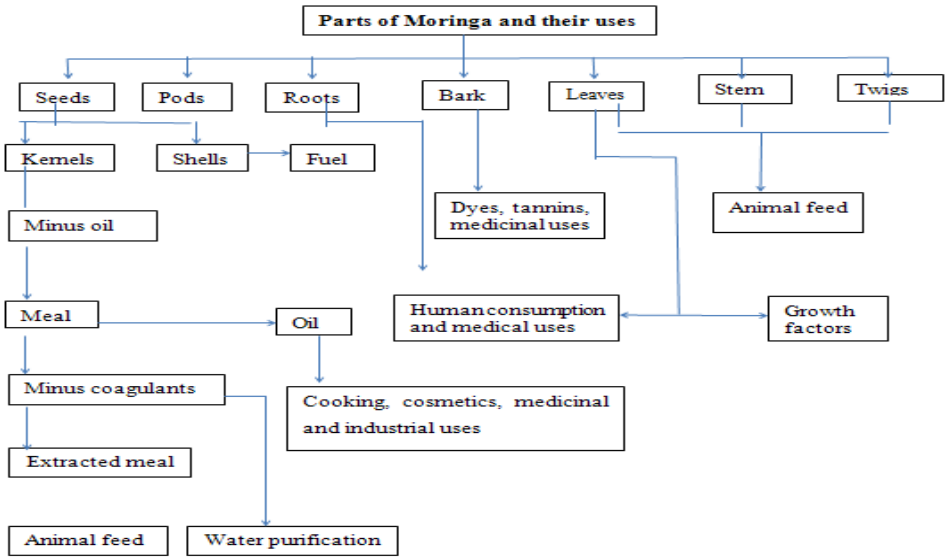

Figure 1: Multiple uses of Moringa
Source: Foidl *et al*. (2001)

Moringa oleifera trees are useful for alley cropping as they have a loose canopy, which prevents excessive crop shading. Foliage can be regularly pruned and left in the field to improve soil fertility or fed to livestock in a cut-and-carry system. The leaves are highly nutritious and contain significant quantities of vitamins (A, B and C), calcium, iron, phosphorus and protein (Murro *et al*., 2003). Furthermore, heavy metals such as mercury,

arsenic and cadmium which are potentially toxic are absent from the leaves of *M. oleifera*, thus making their incorporation into poultry diet safe (Donkor *et al*., 2013).

Other uses of *M. oleifera* to include construction (growth factors, pole and fibre), fuel wood, ornamental (hedge and shade) and medicinal (Maroyi, 2006). Chollom *et al*. (2012) investigated the effect of aqueous seed extract of *M. oleifera* against Newcastle disease virus (NDV) using an *in ovo*assay and found that an increase in extract concentration was directly proportional to virus death and inversely proportional to production of antibody against NDV. These findings have clearly demonstrated that *M. oleifera* seed extract has nutritional value, as well as, strong antiviral activity against NDV *in ovo*.

Inclusion of Moringa in chicken diets

Chickens will not voluntarily consume Moringa leaves or Moringa leaf powder. However, about half the protein content can be extracted from the leaves in the form of a concentrate that can be added to chicken feed (Price, 2007). According to Fuglie (2009), the nutrient value of Moringa leaves can be increased for chickens through the addition of phytase to break down phytate leading to increased absorption of phosphorus. Phytase should be simply mixed with the leaves without heating. If uncontrolled, raw Moringa in poultry diets can be dangerous because of high bio-availability of protein; therefore particular care must be taken to avoid excessive protein intake (Gaia, 2005). Moringa is not only concentrated in nutrients, but in the raw form, it seems to reduce the activity of pathogenic bacteria and moulds and improves the digestibility of other foods, thus helping chickens to express their natural genetic potential (Gaia, 2005). Ayssiwede *et al*. (2011) assessed the effects of MOLM inclusion in poultry diets on growth performances, carcass and organs' characteristics and economics results of growing indigenous Senegal chickens and found that MOLM inclusion in the diets up to 24% did not cause any adverse impact on live body weight, average daily weight gain, feed conversion ratio (FCR), mortality, carcass and organs characteristics in birds compared to their controls.

According to Limcangco-Lopez *et al*. (1989), Moringa fed in high quantities (7.5 and 10%) to one-week old chicks results in reduced growth, indicating that higher levels of Moringa in chick diets has a detrimental effect on chick growth. Onu (2011) in Nigeria investigated the effects of MOLM on the performance and blood chemistry of starter broilers and found that MOLM could be included at 7.5% in broiler diets without any deleterious effect on performance and blood characteristics of broilers. The Golden Valley Agricultural Research Trust (2010) in Zambia incorporated 1 kg MOLM into broiler diets and reported reduced feed intake in the first two weeks. Thereafter, improvement in feed intake was observed which resulted in increased body weight and cock activities. Paguia *et al*. (2012) fed *M. oleifera* leaf and twig powder (MLTP) to force molted hens and assessed their performance. The authors found no effect of MLTP on feed intake, feed efficiency, egg sensory evaluation (egg flavour and egg acceptability score) but reported significant effect on egg weight and feed cost per kilogramme of eggs produced. In another study, the influence of MOLM on growth performance of broilers was assessed and treatment was found to have no effect on average cumulative feed consumption, final live weight, FCR, feed cost per kilogramme of broiler produced, and income over feed and chick cost.

A study by Zanu *et al*. (2011) found that *M. oleifera* when partially used to replace fish meal may hamper growth rate of broiler chickens. In Botswana, Kwedibana (2008) evaluated the effects of MOLM at 10% inclusion level on the growth rate of broilers and found that commercial broiler diet significantly ($P<0.05$) promoted higher weight gain (1.04 kg) than MOLM. Feed intake was also higher for birds fed commercial diets than those on MOLM. On the other hand, FCR was higher for birds on MOLM than those fed commercial diets.

In Zimbabwe, Gadzirayi *et al*. (2012) investigated the effects of supplementing soya bean meals with MOLM as a protein source in poultry and found no significant differences in feed

intake of broilers, however, significant differences in FCR were noted. It was concluded that inclusion of MOLM as protein supplement in broiler diets at 25% promoted more growth than commercial diets. Portugaliza and Fernandez (2012) supplemented Cobb broiler diets with varying concentrations of *M. oleifera* aqueous leaf extract (MoALE) through drinking water and found that at 90 ml MoALE, feed intake of broilers was consistently lower than that of control group (commercial diet). The live weight of broilers given 30 ml, 60 ml and 90 ml MoALE were significantly higher than the control group. The MoALE treated broilers were more efficient converters of feeds into meat than the control group. Kakengi *et al*. (2007) in Tanzania investigated the effect of MOLM as a substitute for sunflower seed meal on performance of laying hens and found that MOLM could be used as a source of plant protein at 10% inclusion level in the diet. The authors mentioned that in areas where MOLM can be obtained for free and quality of eggs fetch higher premium price, inclusion of MOLM at 20% is highly recommended. The study concluded that MOLM could be used as a source of plant protein since it was highly accepted by the birds even at high dietary inclusion levels. Another study by Abou-Elezz *et al*. (2011) assessed the effects of 0%, 5%, 10% and 15% MOLM inclusion levels on egg production and quality traits of Rhode Island Red hens and reported that MOLM linearly decreased the egg laying rate (60.00, 59.72, 56.13, and 51.87 %) and egg mass and had a quadratic effect on the feed intake (111.15, 111.93, 107.08 and 100.47g/hen/d) when including 0, 5, 10, and 15 % of MOLM, respectively. The authors concluded that MOLM could be acceptable as sustainable feed resource up to 10 % in laying hen diets. Similarly, Tesfaye *et al*. (2012) in a 56 days fed broilers diets containing five inclusion levels of MOLM (*i.e.*, 0%, 5%, 10%, 15% and 20%) and reported significantly higher DM intake, CP intake, body weight and average daily gain for 0% MOLM (control) than other treatments. The authors concluded that MOLM could replace soybean in poultry diets up to 10% inclusion level in the total ration of broilers suggesting that the shrub has some potential in poultry feeding.

Banjo (2012) investigated the effects of inclusion of four levels (*i.e.*, 0%, 1%, 2% and 3%) of MOLM on growth performance of Anak 2000 strains of broilers and found that inclusion of 2% significantly enhanced weight gain. It was found that inclusion of MOLM did not significantly enhance feed intake and feed conversion. Furthermore, the effect of MOLM inclusion in cassava chip based diets fed to broiler chickens was studied in Nigeria and a reduction in performance with increasing inclusion level of MOLM above 5% was observed (Olugbemi *et al*., 2010a). It was concluded that broilers could be safely fed cassava-based diets containing MOLM at a maximum level of 5% without deleterious effects. In a related study, Olugbemi *et al*. (2010b) found that MOLM can be safely included in cassava-based layer diets up to 10% without negatively affecting productivity. These results suggest that the inclusion level of MOLM is lower for broilers compared to layers. In another study, Olugbemi *et al*. (2010c) investigated the potential of MOLM as a hypocholesterolemic agent using layers fed cassava-based diets and reported that *M. oleifera* possesses hypocholesterolemic properties and that it can be included in layers diets to facilitate reductions in egg cholesterol content.

CONCLUSIONS

Dietary inclusion levels of 5 to 20% MOLM in broiler diets and 10% in layer diets have been found to improve bird performance in terms of growth rate and egg production (including egg size). However, if MOLM can be obtained for free and the price of eggs is high, the inclusion level of MOLM can also be increased to 20% in layer diets. The results from previous researches indicate that MOLM could partially replace soybean meal and sunflower seed cake as a protein source in diets for chickens.

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Occurrence of Earthworms in Relation to Soil TC, TOC, TIC in Benghazi, Libya

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ABSTRACT

Benghazi city is the second biggest city in Libya and getting bigger promptly. The city surrounded by farms. However, urban invasion has decreased the agriculture area a lot. In this study, survey on earthworm and TOC around the city has been done. Existing of earthworm is highly related to TOC around the city. Earthworms around the city were in Bouatni ≥ Jarotha ≥ El-Guarsha ≥ Hawari soil. The study show decline in the species in Hawari area. Four species of earthworm were identified around Benghazi city. These were *Aporrectodeatrapezoides*, *Aporrectodea*rosea, *Eiseniaandrei*, and Microsolex*dubius*. *A.* trapezoids formed the dominant and most widespread species of Benghazi.*E. Andrei* was new record in the area. This can lead to using earthworms as bioindicators, which appears to be a useful way to classify soil quality. Harsh environmental conditions and low organic matter may not only limit reproduction but the survival of adult earthworms from year to year.

Keywords: Benghazi, Earthworm, Soil, TOC.

INTRODUCTION

Benghazi (32_10¢N, 20_06¢E), the second largest city in Libya, is colonized by some invertebrates such as earthworms, which contribute much to the soil fertility. Moderate information is available on their distribution and ecology around Benghazi city (Nair et al, 2005). Soil is the region on the earth's crust where geology and biology meet, it is the land surface that provides a home to plant and microbial as well as invertebrates (Pelzer et al., 1993). Soil samples differ in their content depending on climate, soil origin, composition and human activities (Hashem and Al-Obaid, 1996).

Soil organisms are among the major components of soil biomass and play important roles in maintaining the structure and fertility of soil. Invertebrate-mediated processes such as drainage, aeration, and incorporating and degrading organic matter are important in improving soil quality (Barber *et al.,* 1998).There are billions of organisms that make up the

soil food web. These include bacteria, fungi, protozoa, nematode, and invertebrates. Each type of organism plays an important role in keeping the soil healthy. Earthworm is considered as a domain soil organism widely distribution worldwide. Earthworm constitute 60-80% of the terrestrial invertebrate's biomass and play a critical ecological role in soil specially in structuring and increasing the nutrient content of the soil (Connor, 1988).Earthworms are known to play important roles in soil profile development, nutrient cycling, and plant productivity where their population densities are high.Play an important role in decomposition processes by the fragmentation of litter material and stimulating and/or ingesting fungi and bacteria that are very important in the cycling of nutrients (Culyand Berry, 1995). Our aims were to survey earthworm distribution around Benghazi city and compared earthworm distribution with TC (includes both organic and inorganic sample constituents).

MATERIAL AND METHODS

Four stations located within the municipality of Benghazi were selected for the study (Fig. 1). These stations were (1) Bouatni,, (2) El-Guarsha, (3) Jarotha, and Hawari (4), they were categorized into three different habitats of earthworms and Isopoda. These were (i) Clayey loam soil, lemon, olive, guava and orange farm (stations 1) , (ii) Loamy sand soil, rose and flower garden (station 2), (iii) Silt clay soil, plain landscape with wild grasses and Pomegranate and olive plants having medium-sized trees forming canopy (station 3), (iv) rose and flower garden (station 4). Soil, Earthworms and Isopoda were sampled during in March 2012, following these steps a plot of 20 x 20 cm replicates ten times were measured within the survey site 10 x 10 m, of each station with two substations. A ditch of 10 cm deep was dug in the plot and the soil organisms were removed and spread on a white plastic tray, and hand-sorted removing earthworms, Isopoda as they were found. The earthworm and isopods were rinsed in distilled water for a few seconds to remove particles of soil from the body surface and placed in Petri. "Identification and taxonomic assignment was performed using the available detailed studies on earthworm taxonomy and distribution for the whole of France (Bouché, 1972), Hungary (Csuzdi and Zicsi, 2003) and Great Britain (Sims and Gerard, 1999)."

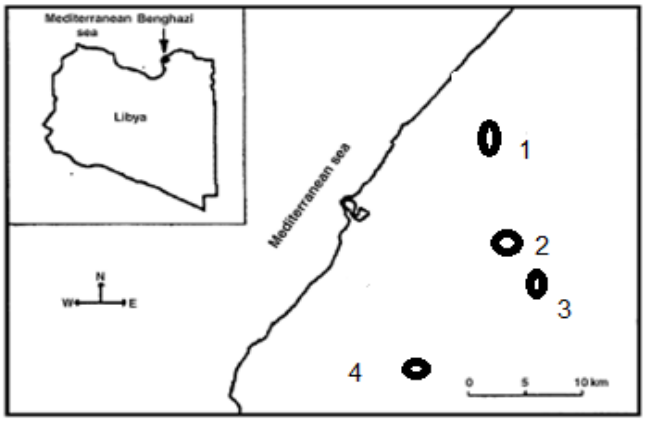

Figure 1. Location of study stations in Benghazi (up to down)
(1) Bouatni, (2) Hawari (3) El-Guarsha, and (4) Jarotha.

RESULTS AND DISCUSSION

Four species of earthworm were identified around Benghazi city (Fig.1). These were *Aporrectodeatrapezoides*, *Aporrectodea*rosea, and *Eiseniaandrei* belonging to the family

Lumbricidae, and Microsolex*dubius* belonging to the family Microscolecidae (Table 1). Out of the four species, *Aporrectodeatrapezoids* formed the dominant and most widespread species of Benghazi and this earthworm was present in all stations.

Table 1. Earthworm species sampled from different stations around Benghazi

stations	Our samples	Nair *et al*., 2005, samples
Bouatni	*Aporrectodea trapezoids, Aporrectodearosea, Eiseniaandrei*	*Microscolexdubius, A. trapezoides, A. rosea*
El-Guarsha	*Aporrectodea trapezoids*	*A. caliginosatrapezoides*
Jarotha	*Aporrectodea trapezoids, Micro scolexdubius, Aporrectodearosea*	*Non*
Hawari	Non	*caliginosatrapezoides*

This was followed by *A. rosea* sampled from two stations (Bouatni and Jarotha). Meanwhile, *Eiseniaandrei* were sampled from just one station (Bouatni) as well as *M. dubius*were sampled from one station Jarotha. *A. caliginosa* trapezoids formed the dominant and most widespread species of Benghazi. This was followed by *A. rosea* sampled from two and *M. dubius* from one and *E andrei* from one stations. Earthworm diversity tended to be low with one to three species present within location. Low earthworm species diversity within a site is not uncommon. Most earthworm diversity studies report the presence of between two and five species at any one location (Lee, 1985). This survey highly agreed with previous survey which done in 2002 by (Nair *et al*., 2005).

However, *E. andrei*was new record in the area. Also, three species of earthworm *A. trapezoids*, *M. scolexdubius*, *Aporrectodearosea*were recorded in Jarotha area, this area was not included in the previous study. In the Hawari stations no earthworm were found However, Nair and his group found the *A. trapezoids*in the area which was not confirmed by our survey, this could mean decline in the earthworm population in the area. Bouatni soil seem to be more suite soil to earthworm species and biodiversity were higher than other soil followed be Jarotha station, these two stations are remain in agriculture use and far from urban invasion, so, they are rich of nutrient. This due to high concentration of TOC compared to other location. Exist of the earthworm highly related to TOC concentration (Fig.2).

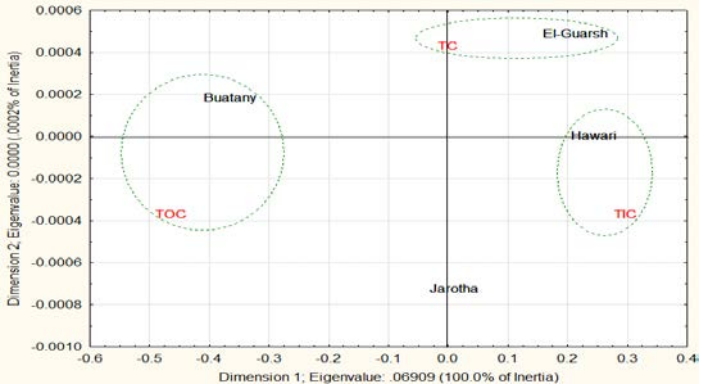

Figure 2. Correspondence analysis plot for. A significant difference exists between the three analyses within the areas.

Higher numbers of species were found in Bouatni than in other sites, this appeared to be related to higher organic matter content in site resulting from fertilizer additions and leaf litter inputs, which improve earthworm food quality, quantity, and soil environmental conditions. This is consistent with previous research that established correlations between earthworm diversity and soil litter quality (Sinha *et al*., 2003).These species play a paramount role in the decomposition of soil litter and their distribution correlates with that of the TOC. Organic

matter contains nutrients necessary for earthworm survival, and soils lacking organic matter do not typically support large earthworm populations (Lee, 1985; Edwards and Bohlen, 1996). Jordan *et al.*, (2000) found that earthworm density decreased in El-Guarsha as well as Hawariwhere organic matter had been decreased due to reduction in the agriculture area and invasion of urban, and attributed the population decline to decreased nutrient availability. The low organic C content in El-Guarsha as well as Hawarisite soils did not appear to provide sufficient nutrients to support earthworm populations similar in density to those in Bouatnior Jarothasites.

CONCLUSION

This survey show density of earthworm highly related to TOC, and urban invasion cause decrease in TOC. This can lead to using earthworms as bioindicators,which appears to be a useful way to classify soil quality. Our recommendation to use soil around Benghazi city in agriculture purpose can be Bouatni ≥ Jarotha ≥ El-Guarsha ≥ Hawari soil. This study were put more emphasis on the pervious study which done in 2002, and *Aporrectodea trapezoids* were domain species and *Eisenia Andrei were recorded as* new species. Moreover, Jarotha area, has not been considered in earthworm survey, and was rich of some species that not found on other area. Also, put indication about decline of earthworm in Hawari area. Finally, harsh environmental conditions and low organic matter may not only limit reproduction but the survival of adult earthworms from year to year.

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Influence of Some Factors on Composition of Dromedary Camel Milk in Sudan

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ABSTRACT

The present study was carried out to investigate the impact of management systems, breeds, parity and stage of lactation on milk composition of Sudanese Arabi camels. Samples of camel milk were collected from 120 healthy she-camels from three different indigenous breeds (Kenani, Nefidia and Butana) in two different management systems (traditional nomadic system and semi intensive system). The milk samples were from camel of 5 parity numbers (1-5 parities) and 4 lactation stages. The highest significant percentages of camel milk fat, protein, lactose, total solids (TS) and solids non fat (SNF) were recorded for the camel in the traditional nomadic system (Nefidia and Butana) followed by the semi intensive system (Kenana breed). Moreover, the mean protein, lactose, TS and SNF values of camel milk were significantly higher during the first stage of lactation, while the mean for fat was significantly high during the third stage of lactation. Fat, protein, lactose, TS and SNF values of camel milk were higher in the fifth parity. Camel reared in the traditional nomadic system (Nefidia and Butana breed) and semi intensive system (Kenana breed) had significantly high content of milk fat compared to their counterparts. However, non significant differences in fat percentage during the fourth parity were observed. The TS and SNF of camel milk were significantly high at the first stage of lactation in comparison with the second and fourth ones. The results indicated that variations in chemical composition of camel milk were mainly attributed to factors such as management systems, breed, parity number and stage of lactation.

Keywords: Management systems, chemical composition, camel milk.

INTRODUCTION

Dromedary camels (*Camelus dromedarius*) can survive and produce considerable amount of milk during recurrent and prolonged hot and dry environment (Bekele *et al*., 2011). Camel milk is considered one of the most valuable food sources for nomadic people in arid and semi arid areas and has been consumed for centuries due to its nutritional values and medicinal

properties (Kenzhebulat *et al*., 2000; El Zubeir and Nour 2006; Farah *et al*., 2007; Lorenzen *et al*., 2011). It is considered to have anti-cancer (Magjeed, 2005) and anti-diabetic (Agrawel *et al*., 2003; Agrawel *et al*., 2005) properties. The high content of unsaturated fatty acids of the camel milk may enhance its overall nutritional quality (Karray *et al*., 2005; Konuspayeva *et al*., 2008; Ayadi *et al*., 2009).

Camel milk composition was found to be less stable than other species such as bovine. Previous findings pointed out that the variation in camel milk composition could be attributed to many factors such as analytical measurement procedures, geographical locations, feeding conditions, type of samples and breeds in addition to other factors including milking frequency, stage of lactation and parity numbers (Iqbal *et al*., 2001; Faye *et al*., 2008; Ayadi *et al*., 2009; Al-Haj and Al-Kanhal, 2010; Hammadi *et al*., 2010; Aljumaah *et al*., 2011). However, geographical origin and seasonal variations were found to be the most effective factors on camel milk constituents and chemical composition in production systems (Shuiep *et al*., 2008). The mean values of camel milk composition (%) reported over the past 30 years were: 3.5±0.1; 3.1±0.5; 4.4±0.7; 0.97±0.07 and 11.9±1.5 for fat, protein, lactose, ash, and total solids, respectively (Al-Haj and Al-Kanhal, 2010).

The camel population in Sudan is about 4.623 millions heads of different indigenous breeds (MARF, 2011). Indigenous camels in Sudan can be classified into different ecotypes or breeds including: Kenani, Butana, Lahawee and others (Wathig *et al*., 2007). Three major production systems: traditional nomadic, semi nomadic and semi intensive systems are practiced in Sudan (Shuiep *et al*., 2008; Ishag and Ahmed, 2011; Eisa and Mustafa, 2011). The composition of camels' milk had been studied under different conditions (El-Amin and Wilcox, 1992; Mehaia *et al*., 1995; Babiker and El Zubeir, 2014). However, there is limited information about the factors affecting milk composition of camels in Sudan. Therefore, the aim of this study was to investigate the effects of management systems, breed, parity number and stages of lactation on the camel milk composition of Sudanese Arabi camel.

MATERIALS AND METHODS

Collection of milk samples

Camel milk samples were collected from three different areas in central Sudan (Sinnar; Moya mountain, and Gezira State; Al Neb; Al Butana) and Khartoum State (Khartoum North locality and Eastern Nile locality), during July 2013 to August 2013. A total of 120 milk samples were collected from 120 healthy she-camels of three indigenous breeds; Kenani and Nefidia and Butana in traditional nomadic system and semi intensive system, respectively. In total 120 bulk milk samples (40 samples/system) were collected in dry clean bottles (60 ml). The samples were labelled and transferred in an icebox to the Dairy Chemistry laboratory of the Faculty of Animal Production, University of Khartoum for the chemical analysis. According to parity, the collected samples were divided into five categories; first, second, third, fourth and fifth. The stage of lactation was also divided into four stages; first (from birth to 3 months), second (from 4 to 6 months), third (from 7 to 9 months) and fourth (from 10 to the end of lactation) stages.

Management systems of camel

In traditional nomadic system, the camels spend all the time in the pasture with restricted access to water. In semi intensive system, the entire herd was kept in pens all the year, and the daily ration consists of a mixture of groundnut cake, *Sorghum biocolor* (Feterita) and *Sorghum biocolor* (Abu70) in addition to continuous water supply. In traditional nomadic system, camels were hand milked three times a day (6:30 am, 1:00 pm and 7:00 pm) and in

semi intensive system the camels were hand milked three times (6:00 am, 1:30 pm and 7:00 pm).

Chemical analysis

Each milk samples was analysed for the content fat, protein, solids not fat, total solids, lactose content and density. The content of milk components were measured twice using Lactoscan milk Analyzer (Milkotronic LTD, Europe) according to the manufacturer's instructions.

Statistical analysis

The data were analysed using the General Linear Model (GLM) procedure in SPSS (Statistical Package for Social Sciences, v.17). Differences between means were separated by Duncan's Multiple Range Test (DMRT) when the significant differences existed.

RESULTS AND DISCUSSION

Results revealed that camel milk composition was significantly ($P<0.05$) affected by the management systems (Table 1, 2 and 3). The highest percentage of fat (4.59), protein (3.53), lactose (4.81), total solids (13.62) and SNF (8.99) were recorded in milk of camel kept in the traditional nomadic system (Nefidia breed). In contrast, these components showed the lowest values in milk of camel kept under the traditional nomadic system (Butana breed). The fat content was higher in milk of camel managed under the traditional nomadic system (Nefidia breed) than semi intensive system (Kenana breed) and traditional nomadic (Butana breed) management systems (4.59% vs. 4.20% and 3.36% respectively). The lower values of camel milk fat content found in the traditional nomadic (Butana breed) system might be due to lack nutrient supplements in comparison to those in the semi intensive system. The fat percentage of Nefidia camel milk recorded the highest value (4.59%) than Kenana and Butana camel milk. Konuspayeva *et al*. (2009), Al-Haj and Al-Kanhal (2010); Babiker and El Zubeir (2014) reported that camel milk composition was influenced by regional differences including feeding conditions. These results partly agreed with those reported previously in Bedouin camels under semi nomadic system (Guliye *et al*., 2000). On the other hand, the obtained results disagreed with the results reported by Haddadin *et al*. (2008) where the milk composition in camels was found to be independent of the grazing system. Variations observed in camel milk composition could be attributed to several factors including management systems (Bekele *et al*., 2008; Shuiep *et al*., 2008; Riyadh *et al*., 2012; Babiker and El Zubeir, 2014), geographical locations, feeding conditions (Khaskheli *et al*., 2005; Bekele *et al*., 2008), seasons (Shuiep *et al*., 2008; Haddadin *et al*., 2008 and Riyadh *et al*., 2012), stage of lactation and calving number (El-Amin *et al*., 2006; Zeleke, 2007; Riyadh *et al*., 2012).

Significant differences among the three studied breeds in the chemical composition of camel milk were observed (Table 1, 2 and 3). This result agreed with those of other researchers (Alshaikh and Salah, 1994; Gaili *et al*., 2000; Khaskheli *et al*., 2005; Konuspayeva *et al*., 2009; Ereifej *et al*., 2011; Babiker and El Zubeir (2014) who reported that camel milk components were significantly affected by breed of lactating camels. The Nefidia camel's milk had the highest contentsof protein, lactose, TS and SNF (3.53%, 4.81%, 13.62% and 8.99%, respectively). These results are consistent with those of Babiker and El Zubeir (2014) who found similarities between camel milk components of Kenani and Anafi but reported differences in these components between these two camel breeds.

Table 1: Means± S.E of camel milk components (%) as influenced by Semi-intensive system, Kenana breed, Parity order and stages of lactation

Parity Number	Stages of Lactation/ Month	Fat	Protein	Lactose	T. S	SNF	Density (mg/dl)
(1-2)	(1-2)	3.60^{a}±0.19	3.34^{a}±0.05	4.60^{a}±0.06	12.19^{a}±0.25	8.65^{a}±0.13	1.030^{a}±0.001
	(4-6)	3.79^{a}±0.38	3.23^{a}±0.09	4.43^{a}±0.12	12.17^{a}±0.49	8.29^{a}±0.25	1.029^{a}±0.003
	(7-9)	5.32^{a}±0.25	3.22^{a}±0.06	4.34^{a}±0.08	13.48^{a}±0.33	8.20^{a}±0.17	1.027^{a}±0.002
	(≤10)	4.51^{a}±0.36	3.27^{a}±0.09	4.45^{a}±0.11	12.92^{a}±0.47	8.41^{a}±0.24	1.028^{a}±0.002
3	(1-2)	4.74^{a}±0.41	3.27^{a}±0.09	4.43^{a}±0.13	13.07^{a}±0.54	8.34^{a}±0.28	1.028^{a}±0.003
	(4-6)	3.78^{a}±0.24	3.20^{a}±0.06	4.39^{a}±0.08	11.99^{a}±0.31	8.22^{a}±0.16	1.028^{a}±0.002
	(7-9)	3.44^{a}±0.41	3.04^{a}±0.09	4.18^{a}±0.13	11.35^{a}±0.54	7.82^{a}±0.28	1.047^{a}±0.003
	(≤10)	4.44^{a}±0.32	3.39^{a}±0.08	4.62^{a}±0.10	13.11^{a}±0.42	8.67^{a}±0.21	1.030^{a}±0.002
4	(1-2)	6.25^{a}±0.41	3.29^{a}±0.09	4.38^{a}±0.13	14.46^{a}±0.54	8.30^{a}±0.28	1.027^{a}±0.003
	(4-6)	3.20^{a}±0.72	3.13^{a}±0.17	4.35^{a}±0.23	11.31^{a}±0.93	8.11^{a}±0.48	1.028^{a}±0.005
	(7-9)	3.14^{a}±0.51	3.26^{a}±0.12	4.50^{a}±0.16	11.54^{a}±0.66	8.40^{a}±0.34	1.029^{a}±0.004
	(≤10)	3.87^{a}±0.72	3.63^{a}±0.17	4.98^{a}±0.23	13.19^{a}±0.93	9.31^{a}±0.48	1.032^{a}±0.005
5	(1-2)	2.15^{b}±0.69	2.10^{b}±0.14	3.59^{b}±0.27	9.99^{b}±0.69	7.39^{b}±0.59	1.015^{b}±0.001
	(4-6)	5.52^{a}±0.72	3.34^{a}±0.17	4.49^{a}±0.23	14.00^{a}±0.93	8.49^{a}±0.48	1.028^{a}±0.005
	(7-9)	3.33^{a}±0.72	3.28^{a}±0.17	4.54^{a}±0.23	11.80^{a}±0.93	8.47^{a}±0.48	1.030^{a}±0.005
	(≤10)	2.10^{b}±0.69	2.50^{b}±0.14	3.30^{b}±0.27	8.89^{b}±0.69	7.10^{b}±0.59	1.014^{b}±0.001

$^{a-b}$ Means with different letters in the same superscript are significantly different at ($p \leq 0.05$)

Table 2 showed the effect of traditional nomadic system on Butana breed, parity number and stages of lactation on camel milk constituents. Results showed significantly ($P<0.05$) differences in fat, protein, lactose, TS and SNF of camel milk in the different parities. Where camel in the first stage of lactation revealed high mean values protein, lactose, TS and SNF percentages for milk (3.49±0.03, 4.77±0.04, 13.14±0.16 and 8.97±0.07%, respectively). Meanwhile, the mean values of milk constituents were lower in the subsequent parity.

Table 2: Means± S.E of camel milk components (%) as influenced by Traditional Nomadic system, Arabi (Butana) breed, parity order and stages of lactation

Parity Number	Stages of Lactation /Month	Fat	Protein	Lactose	T. S	SNF	Density (mg/dl)
(1-2)	(1-3)	3.89^{a}±0.36	3.28^{a}±0.09	4.54^{a}±0.11	11.83^{a}±0.47	8.45^{a}±0.24	1.029^{a}±0.002
	(4-6)	2.08^{b}±0.27	1.99^{b}±0.07	3.69^{b}±0.23	8.59^{b}±0.36	6.59^{b}±0.39	1.019^{b}±0.001
	(7-9)	2.13^{b}±0.27	2.00^{b}±0.07	3.59^{b}±0.23	8.25^{b}±0.36	6.31^{b}±0.39	1.018^{b}±0.001
	(≤10)	3.48^{a}±0.36	2.96^{a}±0.09	4.07^{a}±0.11	11.12^{a}±0.47	7.61^{a}±0.24	1.027^{a}±0.002
3	(1-3)	3.89^{a}±0.29	3.53^{a}±0.07	4.85^{a}±0.09	13.06^{a}±0.38	9.18^{a}±0.20	1.032^{a}±0.002
	(4-6)	2.55^{a}±0.41	3.33^{a}±0.09	4.64^{a}±0.13	11.17^{a}±0.54	8.62^{a}±0.28	1.031^{a}±0.003
	(7-9)	2.63^{a}±0.41	3.21^{a}±0.09	4.48^{a}±0.13	10.94^{a}±0.54	8.31^{a}±0.28	1.029^{a}±0.002
	(≤10)	3.22^{a}±0.29	2.88^{a}±0.07	3.90^{a}±0.09	10.16^{a}±0.38	7.40^{a}±0.20	1.025^{a}±0.003
4	(1-3)	2.98^{a}±0.51	4.16^{a}±0.12	5.79^{a}±0.16	13.28^{a}±0.66	10.78^{a}±0.34	1.038^{a}±0.004
	(4-6)	1.29^{b}±0.42	2.56^{b}±0.06	3.89^{b}±0.26	9.29^{b}±0.66	7.01^{b}±0.21	1.020^{b}±0.001
	(7-9)	1.22^{b}±0.39	2.40^{b}±0.06	3.70^{b}±0.23	9.10^{b}±0.38	6.59^{b}±0.19	1.021^{b}±0.001
	(≤10)	3.05^{a}±0.29	3.14^{a}±0.07	4.33^{a}±0.09	11.13^{a}±0.38	8.08^{a}±0.20	1.028^{a}±0.002
5	(1-3)	4.28^{a}±0.72	4.21^{a}±0.17	5.78^{a}±0.23	15.10^{a}±0.93	10.82^{a}±0.48	1.038^{a}±0.005
	(4-6)	3.45^{a}±0.72	3.67^{a}±0.17	5.07^{a}±0.23	12.92^{a}±0.93	9.47^{a}±0.48	1.033^{a}±0.005
	(7-9)	3.72^{a}±0.41	3.20^{a}±0.09	4.39^{a}±0.13	11.93^{a}±0.54	8.21^{a}±0.28	1.028^{a}±0.003
	(≤10)	5.13^{a}±0.72	3.71^{a}±0.17	5.04^{a}±0.23	14.62^{a}±0.93	9.48^{a}±0.48	1.032^{a}±0.005

$^{a-b}$ Means with different letters in the same superscript are significantly different at ($p \leq 0.05$)

However, no significant differences in the camel milk constituents during the first, second and third stages of lactation were found. In the fourth stage of lactation, milk constituents were significantly decreased. Similarly the data revealed non significant differences in fat content of camel milk with the variation in the parity order, however there was a slight decreased in the fat content in the first (4.27±0.12%) and fifth parity (4.29±0.22%). In contrast, Zeleke (2007) mentioned that the effect of parity on fat content of camel milk was

significant. The camel milk in the fifth parity had the highest fat content (4.29±0.22%). The highest level of lactose was observed in the fifth parity (4.79±0.08%). This result is concordant with those of Zeleke (2007); Riyadh *et al*. (2012) who reported that the highest lactose content in camel milk was recorded in the first stage of lactation. This observation probably explains the common understanding among camel milk producers that camel milk is sweeter during first lactation than other subsequent lactations (Riyadh *et al*., 2012).

Table 3: Means± S.E of camel milk components (%) as influenced by Traditional Nomadic system, Arabi (Nefidia) breed, parity order and stage of lactation

Parity Number	Stages of Lactation/Month	Fat	Protein	Lactose	T. S	SNF	Density (mg/dl)
(1-2)	(1-3)	5.18^{a}±0.41	3.49^{a}±0.09	4.71^{a}±0.13	14.08^{a}±0.54	8.90^{a}±0.28	1.034^{a}±0.003
	(4-6)	4.94^{a}±0.29	3.66^{a}±0.07	4.97^{a}±0.09	14.23^{a}±0.38	9.34^{a}±0.20	1.034^{a}±0.002
	(7-9)	4.52^{a}±0.41	3.60^{a}±0.09	4.90^{a}±0.13	13.73^{a}±0.54	9.21^{a}±0.28	1.031^{a}±0.003
	(≤10)	4.93^{a}±0.72	3.80^{a}±0.17	5.17^{a}±0.23	14.64^{a}±0.93	9.71^{a}±0.48	1.031^{a}±0.005
3	(1-3)	4.78^{a}±0.29	3.54^{a}±0.07	4.83^{a}±0.09	13.85^{a}±0.38	9.08^{a}±0.20	1.031^{a}±0.002
	(4-6)	4.56^{a}±0.29	3.42^{a}±0.07	4.67^{a}±0.09	13.31^{a}±0.38	8.75^{a}±0.20	1.030^{a}±0.002
	(7-9)	4.02^{a}±0.41	3.34^{a}±0.09	4.57^{a}±0.13	12.59^{a}±0.54	8.57^{a}±0.28	1.030^{a}±0.003
	(≤10)	3.99^{a}±0.32	3.67^{a}±0.08	5.04^{a}±0.10	13.42^{a}±0.42	9.44^{a}±0.21	1.033^{a}±0.002
4	(1-3)	4.54a±0.29	3.75a±0.07	5.11a±0.09	14.12a±0.38	9.58a±0.20	1.033^{a}±0.002
	(4-6)	3.79^{a}±0.32	3.09^{a}±0.08	4.24^{a}±0.10	11.72^{a}±0.42	7.94^{a}±0.22	1.027^{a}±0.002
	(7-9)	5.12^{a}±0.25	3.56^{a}±0.06	4.82^{a}±0.08	14.16^{a}±0.33	8.71^{a}±0.17	1.031^{a}±0.002
	(≤10)	2.10^{b}±0.24	2.29^{b}±0.09	3.79^{b}±0.10	10.12^{b}±0.31	6.90^{b}±0.25	1.022^{b}±0.001
5	(1-3)	3.10^{b}±0.41	2.10^{b}±0.09	3.69^{b}±0.13	10.39^{b}±0.54	6.90^{b}±0.48	1.022^{b}±0.001
	(4-6)	4.07^{a}±0.51	3.69^{a}±0.12	5.07^{a}±0.16	13.54^{a}±0.66	9.48^{a}±0.43	1.033^{a}±0.004
	(7-9)	4.90^{a}±0.41	3.45^{a}±0.09	4.68^{a}±0.13	13.71^{a}±0.54	8.81^{a}±0.28	1.027^{a}±0.003
	(≤10)	3.00^{b}±0.51	2.26^{b}±0.12	3.59^{b}±0.16	10.00^{b}±0.66	7.00^{b}±0.56	1.021^{b}±0.002

$^{a-b}$ Means with different letters in the same superscript are significantly different at ($p \leq 0.05$)

Camel milk composition was affected significantly ($P<0.05$) by stages of lactation. The protein, lactose, TS and SNF content of camel milk were higher during the first and third stage of lactation (Table 1 and Table 3). The obtained results followed the same trend reported by Alshaikh and Salah (1994), Haddadin *et al*. (2008); Zeleke (2007) who found that values of fat, protein and total solids were highest during the first 6 months of lactation. Camel milk constituents were lower during the second and fourth stage of lactation (Table 2), this may be due to the increase in the milk water content during the last stage of lactation (Riyadh *et al*., 2012). These results confirm those of Gaili *et al*. (2000); Zeleke (2007) who demonstrated that total solids of camel milk decreased from 11.7% in the first stage of lactation to 10.1% by the end of lactation and that fat content of camel milk was gradually decreased with the progress of the stage of lactation.

CONCLUSION

The present study emphasized that the variations in camel milk chemical composition could be attributed to more factors such as production systems, breed, parity number and stages of lactation. The performance of she camels at traditional nomadic system was better in comparison to the other system (semi-intensive). For the future studies, more research conducted to delineate management systems for the camel to in order to improve the milk chemical composition.

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Original Article

Babesiosis in a Four Year Old Friesian–Sokoto Gudali Crossed Bull in Sokoto, Nigeria

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ABSTRACT

Bovine babesiosis is a hemo-protozoan disease diagnosed in a four-year old heavily tick infested Friesian-SokotoGudalli crossed bull. Clinical signs observed were pyrexia (rectal temperature of 40.8^{o}C), anaemia, anorexia, haemoglobinuria, dysuria, salivation and lacrimination. The bull was weak and could not graze. The thin blood smear was positive for both *Babesiabigemina* and *B. bovis*. The bull's condition improved after the administration of the following drugs; diminazineaceturate 7.0 mg/kg, antipyrine 8.6 mg / kg I.M. and application of acaricide, Flumethrin® on the body of the body of the animal. The management of the case was discussed in the paper.

Keywords: Babesiosis, Bull, Four years old, Friesian–SokotoGudali, Crossed.

INTRODUCTION

Babesiosis, or tick fever, is a febrile disease of domestic and wild animal hosts and sometimes human, caused by *Babesia* species; a tick transmitted protozoan parasites (Kuttler, 1988). It is characterized by extensive erythrocyticlysis leading to a haemoglobinuria and if untreated could be fatal. Bovine babesiosis may be caused by any of these two distinct species; the large (*B. bigemina*) and the small (*B. bovis*).

Clinical cases are described as babesiosis while subclinical infections and those recovered from clinical attack are termed babesiasis. Transplacental transmission occurs in *B. bovis*, *B. bigemina* and *B. equi*. (Uilenberg, 1995; Oliveira *et al.*, 2008). The disease is endemic throughout most of sub-Saharan Africa and neighbouring Islands. *B. bigemina* has been reported from central and south America, Europe, Africa, Australia and Asia, while *B. bovis* has been reported from Europe, Africa and Asia. *B. argentina* is reported from south east Asia, Australia, Mexico and Latin America (Guedes*et et al*., 2008).

Babesiosis is not only of economic importance as it causes substantial losses, directly due to mortality, ill-thrift, abortion, loss of milk/meat production and draft power and indirectly through costs associated with its control such as acaricide treatments, purchase of vaccines and therapeutics but also through its impact on international trade (Bock *et al*., 2004). Traditionally, control of babesiosis has relied almost exclusively on vector control by means of intensive application of

costly and toxic acaricides which have certain drawbacks as residues in meat, milk and environmental contamination (Meltzer and Norval, 1993) and thus, a health hazard.

CASE REPORT

On Sunday, the 17th of October, 2011, a four year old SokotoGudali-Friesian Holstein-Cross bull weighing approximately 800kg belonging to the University was presented to the Veterinary Teaching Hospital, UsmanuDanfodiyo University, Sokoto. The complaint was that the animal fell on ground thrice while grazing (once and twice in the morning and afternoon respectively) it was also reported that the animal was off feed and was very dull. The bull was introduced to 22 Gudali heifers having been purchased, to improve the stock of Gudali heifers meant for dairy production. Also kept in the farm were 12 Gudali cattle belonging to the Faculty of Veterinary Medicine, UsmanuDanfodiyo University, Sokoto, 50 Azwak fattening bulls and 120 Red Sokoto goats and 10 Yankasa sheep. The animals were allowed to graze on the rangeland after supplementary feed comprising of rice bran groundnut husk, wheat bran and cotton seed cake are provided after grazing.

The animals were said to have been deticked 6 weeks earlier before presentation. Our **differential diagnoses** werebabesiosis, helminthosis, coccidiosis, rinderpest, hemorrhagic septicaemia, anthrax,while the **Tentative diagnosis** was babesiosis (due to hemoglobinuria which was highly indicative of babesiosis). The **Plan of action** was to take sample for parastitological examination, to take blood sample for haematology, to send tick sample collected from the animal for identification and to commence treatment against babesiosis.

The drugs given were diminazeneaceturate 7.0mg/kg, antipyrin 8.6mg/kg (Samorenil) 7.08 was given I.M.once.

LABORATORY RESULT

Blood sample, thin blood smear revealed *Babesiabigemina* (+++)*[1], *Babesiabovis*(+)*, tick identified based on morphological description and key (Soulsby, 1982; Walker *et al*., 2003) was *Rhipicephalus* (*Boophilus*) *decolaratus*.

Physical Examination/ Clinical Findings

On physical examination, the animal was apparently dull and sluggish. There was excessive salivation and lacrimation. The animal was anorexic, attempt was made to force feed it but it showed inappentance. There was dysuria, with little voided reddish urine. Further examination revealed pale ocular mucus membrane and massive tick's infestation around the perineal, inguinal and axillar region. The vital parameters show rectal temperature of 40.8^{o}C, pulse rate of 96 beats/min, weak and irregular respiratory rate at 42cycles/minute. Problem list included: - anorexia, pyrexia, anaemia, hemoglobinuria, massive tick infestation, anal-haematoma, dysuria, salivation and lacrimaton. Hematology results from parasitology showed packed cell volume 15%.

Confirmatory diagnosis: - *Babesiosis*

Follow up; On Monday, the following week, the animal was deticked with an acaricide;Amitrax®. The animal was active, the hemoglobinuria had reduced considerably, the anal haematoma had regressed markedly and the animal started feeding gradually. The temperature had dropped to 39.2^{o}C, pulse rate was 80 beats/minute and respiratory rate was 25 cycles/min. On Wednesday 20th October, 2011 the animal was found to be feeding well, the anal haematoma had regressed completely, lacrimation and salivation had reduced also the animal was seen grazing with others.

DISCUSSION

This case on babesiosis appears to be among the very few and rare cases in Sokoto, Northwestern Nigeria, though the condition has been reported in some other parts of the country

[1] .* Degree of tick infestation

(Ajayi*et al*., 1982; Larmode, 1986).Babesiosis in cattle in this part of the country is rare but occasionally seen. The indigenous cattle may almost invariably experience milder clinical symptoms to primary infection; this especially observed if naive or immunocompromised cattle are moved to a paddock highly infested with *Rh.* (*Boophillus*) *decolaratus*infestation. Similarly, young calves exhibit a strong innate immunity compared to adult cattle (Goff *et al*., 2001). However, exotic breeds like the Friesian–Holstein or their crosses are more susceptible.

The two important species *B. bigemina* and *B. bovis* are widely spread in tropics and sub tropics. Incidentally, these are the two species identified in our Parasitology laboratory, Faculty of Veterinary Medicine, UsmanuDanfodiyo University, Sokoto. History revealed that tick infestation had been one of the herd health problems on the farm and that therapeutic measures have been put in place using Flumethrin®. *Rhipicephallus* (*Boophillus*) *decolaratus* was the tick found on the body of the bull just as it has been reported in other areas of the world (Friedhoff, 1988).

Clinically, high fever, parasitaemia anorexia, dark coloured urine, haemoglobinemia and haemoglobinuria have been reported earlier in similar cases (Ajayi*et al*., 1982, de Vos and Potgieter, 1994). Treatment depends on early and prompt administration of appropriate drugs. Protection depends on premonition and continued exposure to infected ticks, whereas premonition of babesiosis in enzootic areas depends on the elimination of the tick vector by regular dipping of cattle with acaricide. The survival of Friesians and their crosses has often presented a serious problem to most investors, especially the favorable climatic conditions prevalent in this area that support the growth and the development of tick and perhaps because of lack of enzootic stability.

CONCLUSION

Since Friesian and their crosses are highly susceptible to babesiosis and other haemoparasitic diseases, theyshould be routinely examined and dipped against ectoparasites. Prevention of Babesiosis in enzootic areas depends on elimination of the tick vector by regular dipping of cattle with acaricide

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Effects Of Supplementation with Sycamore Fig (*Ficus Sycomorus*) on Performances of Washera Sheep Fed Natural Pasture Hay and Its Economic Benefit

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ABSTRACT

The experiment was conducted to evaluate the effect of supplementations with F. sycomorus leaf, fruit and their mixtures on intake, digestibility, body weight gain and carcass parameters of sheep fed basal diet hay, and to assess the economic benefit of the supplementation using partial budget analysis. The experiment was carried out at Gish Abay in Sekela Woreda, West Gojjam Zone; using twenty intact male yearling Washera sheep with a mean (±SD) initial body weight of 17.5±0.39kg. The animals were vaccinated against anthrax and pasteurellosis, dewormed and sprayed against internal and external parasites, respectively, before the start of the experiment. Experimental sheep were adapted for 15 days to the treatment feeds. The experiment consisted of digestibility trial of 7 days and feeding trial of 90 days. The experiment was laid out in a randomized complete block design (RCBD) with five blocks consisting of four animals per block based on their initial body weight. Dietary treatments were randomly assigned to one of the four treatment diets within a block. Treatments comprised of feeding natural pasture hay ad libitum (un-supplemented: T_1) or natural hay supplementation with either F. sycomorus leaf (Treatment 2: T2), or F. sycomorus fruit (Treatment 4; T4), or mixture of F. sycomorus leaf and fruit in a ratio of 1:1(Treatment3; T3). The amount of supplements offered was 300 g/day on DM basis. Water and salt were available free choice. Natural pasture hay in the current study contained 8.0% crude protein (CP), 73.1% Neutral detergent fiber (NDF) and 43.6% acid detergent fiber (ADF). Sheep in the un-supplemented treatment consumed higher ($p<0.001$) basal dry matter intake ((581.6 g/day) as compared to supplemented group. However, total DM intake was higher for sheep in the supplemented group (T2-T4) compared to the un-supplemented (control). Supplementation significantly improved digestibility co-efficient of DM, organic matter (OM) ($P<0.001$) and CP ($P<0.001$). Supplementation highly increased ($P<0.001$) final body weight (FBW), feed conversion efficiency (FCE) and average daily gain (ADG). Sheep supplemented with T2 had significantly higher ($P<0.001$) FBW (21.6 kg), FCE (0.062) and ADG (45.1g/day) as compared to the un-supplemented treatment, which had 18.2 kg, 0.01 and 8g/day, respectively. Furthermore, Sheep in T_2 had significantly higher ($P<0.05$) body weight change compared to the un-supplemented. Similar to biological performance, economic analysis also showed that supplementation with T_2 resulted in better return compared to others. Thus, it can be concluded that supplementation in general improved animal performance. Among the supplements, however, T2 is biologically optimum and economically feasible.

Keywords: Average daily gain, partial budget analysis, Economic benefit, Feed Intake, Feed conversion efficiency and Washera sheep.

INTRODUCTION

Ethiopia is home for diverse indigenous sheep populations, which are estimated to be 26.1 million (CSA, 2009). There are about 14 traditional sheep populations in Ethiopia (Solomon *et al.*, 2007). Dangla (Washera) sheep is one of the traditional sheep found in West and East Gojjam Zone of the Amhara National Regional State extending to the south of Lake Tana. Washera sheep weigh about 2.8 and 13.8 kg at birth and weaning; respectively. The growth rate after weaning is comparable and even better than some other indigenous breeds. This indicates the potential of this breed for commercial mutton production for the local and export market (Kassahun and Solomon, 2008).

Despite the large population, productivity of Ethiopian livestock in general is not appreciable, mainly due to technical and non-technical constraints (EARO, 2001). Among the technical constraints, poor nutrition both in terms of quantity and quality, diseases and low genetic potential hinder animal productivity in the country. Feed shortage particularly during dry season; limit the animal output in most part of the country (Alemayehu, 2005). The available feed resources cannot meet the nutritional requirements of animals throughout the year in many parts of the country either due to inadequate supply or quality of the feed (Adugna, 2008). Livestock feed resources in Ethiopia are mainly natural grazing and crop residues, which are low in energy and protein leading to significant limitation in the productivity of sheep (CTA, 1991). Such feed deficiencies causes loses of weight gains made during more favorable periods, while fodder conservation to help eliminate seasonal feed supply fluctuations are rarely practiced (Alemayehu, 1995). As result, the annual off take of sheep is estimated to be 33% (EAP, 2002), with an average carcass weight of 10 kg, which is the second lowest among sub Saharan Africa countries (FAO, 2004). However, these trends of events can be changed if animals are strategically supplemented with available protein and energy sources such as agro industrial by products or multi-purpose trees (MPT). Nevertheless, the use of agro industrial by products is limited to the area where they are produced or economic factors limit their wider use.

This calls for searching for alternative feed resources which could be used as supplement to improve animal performance. Multi-purpose trees are among the alternatives to be employed since it is abundant in different agro-ecological set up and contains higher nutrients. One potential source in the study area, in this regard, is the leaf and fruit of F .sycomorus. F. sycomorus is MPT and belongs to the family of Moraceae which is native to Ethiopia (Orwa *et al.*, 2009). It is available in Amhara National Regional State. F. sycomorus has been identified as feed of cattle, goat and sheep (Teferi *et al.*, 2008). F. sycomorus leaf are valuable fodder in overstocked semi-arid areas where the trees occur naturally and leaf are much-sought fodder with fairly high nutritive value of about 14-17.95% crude protein (CP) and 12 MJ/kg net energy on DM basis (Nkafamiya (2010; Devendra, 1990).

F.sycomorus leaf and petioles are well accepted by West African Dwarf lambs and led to higher levels of apparent digestibility than the other tree species (Anugwa and Okori, 1987). Feeding Ficus fodder to lambs is actively encouraged in Nigeria. Fruit of the plant are round from 2.8-5 cm in diameter conspicuous opening that may break at the one end and with various colours. Makishima (2005) found that F. sycomorus is the most abundant fruit supplier for frugivorous animals in the riverine forest in the semi arid northern Kenya. It is known that chimpanzees use Ficus species fallback sources of feed during the period of fruit scarcity (Fruichi *et al.*, 2001). Ficus fruit are available all the year round in Africa fruiting 3-5 times per year (Kinnaird, 1992).

In Sekela District, where this study was conducted, sheep feed on natural pasture, fallow land grazing and crop residues; where the nutrients supplied by these feed resources are insufficient to meet for maintenance, growth and production requirements of animals. Sometimes farmers in the area purchase protein supplements such as cotton seed

meal and low quality roughage during dry season, but it is not effectively utilized. Moreover, the animals feed on fallen leaf and fruit of F. sycomorus since the tree grows around farm land residence and on the degrading area. In fact the leaf and fruit of tree are important sources of nutrient for small ruminants in the dry season. However, systematic evaluation of the value of F. sycomorus leaf and fruit for sheep has not been well researched in the study area. The current study was, therefore, designed to evaluate effects of supplementation with F. sycomorus leaf, fruit and their mixtures on performances of Washera sheep fed natural pasture hay and to assess the economic benefit of the effect of supplementation with F.sycomorus.

MATERIALS AND METHODS

Study Site

This study was conducted in Sekela Woreda in West Gojjam Administrative Zone, North-Western Ethiopia. The site is located at 466 km North West of Addis Ababa and situated at an altitude ranging from 2013 and 3257meter at sea level. The average annual rainfall of the area was 1738 mm with a bi-modal distribution from February to April and from June to September. The average annual minimum and maximum temperature was 8 and 21°C, respectively (Worldclim, 2009). Sekela has undulating landscape with degraded farmlands. Mixed crop- livestock production was the typical farming system in the Woreda with tree growing (Eucalyptus) as a common practice around farmlands and homesteads. Animal production in the Woreda is vital for the security and survival of large numbers of people. According to Sekela Agricultural and Rural Development Office (SARDO, 2009); the average land holding per household is 0.75 hectare.

Feed Preparation

Leaf of F. sycomorus was harvested by climbing the tree and pruning the branch of tree at the end of the rainy season from communal lands, local farmer's farm yard, and river banks around Gish Abay. The time of harvesting was determined based on the intensity of sunlight that facilitates drying and optimum growth of leaf with which biomass becomes higher. F. sycomorus fruit were also picked from the local tree fruit around Gish Abay. The Collection of fruits was done under the tree plant after the tree fruit ripened (red color). Fruit were fallen by the help of man. The leaf and fruit were air-dried under shade. F. sycomorus leaf's petiole was removed (twigs separating after lopping). The dried leaf was partially crushed and fruit were collected and put into sacks and stored in a well ventilated shade until used at room temperature. Adequate supplies of the experimental feeds were stored for use during the whole study period. The basal feed was natural pasture hay which was purchased from the surrounding farmers. After harvesting the hay was transported to the study sites, stored under shade to maintain its quality and used as a basal diet throughout the experimental period. Hay was manually chopped to the size of about 1-6 cm to minimize selective feeding by the sheep.

Animals and Management

Twenty yearling intact male Washera sheep were purchased from local market of Gish Abay. The animals were quarantined for a period of 21 days. During this period, all sheep were ear tagged for identification purpose, sprayed with acaricides (diazzinole) for external parasites and injections of Ivermectin solution for internal parasite and were vaccinated against common infectious diseases in the area such as pasteurellosis, anthrax and sheep pox based on the prescription of the veterinarian. Initial body weights of the animals was taken on two consecutive weighing after overnight fasting at the beginning of the acclimatization period (17.5$\pm$ 0.39; Mean$\pm$SD), and animals were grouped into five blocks of four animals each based on their initial BW. Sheep were adapted to the experimental feeds for additional

two weeks before commencement of the actual experiment. Experimental sheep were penned individually; the pen equipped with bucket and feed bins which was made from bamboo. Cleaning of pens was done daily before offering the day's feed.
Measured quantities of natural pasture hay were offered for ad libitum consumption allowing 25% refusal. Adjustment of feed offer was made every week. Supplemented sheep were offered supplements twice a day at 08:00 h, and 16:00 h in equal portions. The animals had available free choices to salt block and water during the whole day**.** The leaf and fruit were offered with plastic sheet which was tied to a bamboo stick over the cage and above the feed bins to collect fruit and leaf that may have fallen down and hay were offered with locally available bamboo feed bins.

Experimental Design and Treatments

The Experimental Design used in this experiment was completely randomized block design. Initial weights of the animals were determined by taking the mean of two consecutive weighing after overnight fasting. Based on the design, sheep were blocked into five groups based on initial body weight and treatments were randomly assigned to five animals per treatment. Dietary treatments were randomly assigned to one of the four treatment diets within a block. Treatments were comprised of feeding natural pasture hay ad libitum (un-supplemented: T_1) or natural pasture hay supplementation with either F. sycomorus leaf (Treatment 2: T2), or F. sycomorus fruit (Treatment 4; T4), or mixture of F. sycomorus leaf and fruit in a ratio of 1:1(Treatment3; T3). The amount of supplements offered was 300 g/day on DM basis.

Feeding Trial

Feeding trial was conducted for 90 days. Daily feed offered to the experimental sheep and the corresponding refusals was recorded and measured during the experimentation period to determine daily feed intake. Representative samples of feed offer per batch, and refusal per animal were collected and stored based on type of feed, pooled over the experimental period and sub-sampled for chemical analysis. Daily feed intake of individual sheep was calculated as the difference between the amounts of feed offered and refused. Substitution rate was calculated as the difference between basal diet intake of the un-supplemented and supplemented treatments divided by the amount of supplement offered (Ponnampalam *et al.,* 2004).

Body Weight Measurements

To determine the weight change of animals in the course of the experiment, live weight of each sheep was taken at every ten days intervals after overnight fasting and in the morning before provision of feed and water. Body weight was measured by using suspended salter scale. Body weight changes were determined as a difference between the final and initial body weight. Average daily body weight gain was calculated as the difference between final body weight and initial body weight of the sheep divided by the number of feeding days. Feed conversion efficiency was calculated by dividing the average daily body weight gain to average daily feed intake.

Chemical Analysis

Representative (composite) samples of feed offer and refusal samples collected during the feed trial were milled to pass through a 1mm sieve screen size and analyzed for DM and ash following the procedure of AOAC (1990). Acid detergent fiber (ADF), NDF and ADL components of each ingredient were determined according to the procedures of Van Soest and Robertson (1985). The crude protein was estimated by multiplying N with a nitrogen factor of 6.25.

Partial Budget Analysis

Partial budget analysis was carried out to determine profitability of the current supplementation strategy using Washera sheep. The analysis considered purchase and sale price of the sheep and cost of feed consumed during the experimental period. Market prices of the sheep were assessed in Gish Abay, Sureba Michael Maksegnt and Awi zone local markets and the value of live animals was estimated by person who was involved in sheep trading. Thus the average estimated price of sheep ranged from 265 to 440 ETB. Using the procedure of Upton (1979) net income (NI) would be calculated as the amount of money left when total variable costs (TVC) were subtracted from the total returns (TR).

NI= TR-TVC

The change in net income (ΔNI) was calculated as the difference between change in total return (ΔTR) and the change in total variable costs (ΔTVC).

ΔNI = ΔTR-ΔTVC.

The marginal rate of return (MRR) measures the increases in net income (ΔNI) associated with each additional unit of expenditure (ΔTVC).

MRR= ΔNI / ΔTVC

Statistical Analysis

Feed intake, digestibility, body weight gain and carcass parameters were subjected to analysis of variance (ANOVA) using the general linear model procedure in SAS soft ware (V9) (SAS, 2002). The association between nutrient intake, digestibility and body weight gain was tested using correlation analysis. Treatment means were separated using least significant difference (LSD). The model employed was:

$$\mathbf{Y_{ij} = \mu + t_i + b_j + e_{ij},}$$

Where; Y_{ij} = Response variable
μ = Overall mean
t_i = Treatment effect
b_j = Block effect (initial body weight)
e_{ij} = Random error

RESULTS

Chemical Composition of the Experimental Feeds

The chemical composition of feedstuff used in this study is given in Table 1 below. In the current experiment the CP content of F.sycomorus leaf was 17.9%. The NDF, ADF, ADL, DM and ash content of F. sycomorus leaf on DM basis in this study was 64.6%, 52.5%, 17.4%, 93.2% and 11.9%, respectively. On the other hand, the CP content of the F.sycomorus fruit in the current study was 11.8%. The hay offered to the experimental animals in the current study had CP content of 7.9% with higher NDF and ADF composition.

Feed Intake

The mean daily intakes of DM, OM, CP, NDF and ADF of Washera sheep fed a basal diet of natural pasture hay and supplemented with F. sycomorus leaf, fruit and their mixtures are presented in Table 2. The basal feed DM intake was higher (P>0.05) for sheep fed on T1 diet as compared to sheep in supplemented group (T2-T4).

Table 1. Chemical composition of feedstuff

Feed type	%DM						
	DM	Ash	OM	CP	NDF	ADF	ADL
Feed offer							
Hay	93.2	8.4	92.6	8.0	73.1	54.6	16.3
FSL	93.2	11.9	88.2	17.9	64.6	52.5	17.4
FSF	92.3	5.7	94.3	11.8	35.2	32.7	14.4
1FSL:1FSF	92.7	8.8	91.3	14.9	49.9	42.6	15.9
Refusal							
Hay (T1)	93.4	10.8	89.2	3.8	80.0	59. 2	16.8
Hay (T2)	93.5	9.3	90.7	4.8	81.3	55.9	16.3
Hay (T3)	93.4	8.9	91.1	5.1	78.7	58.3	17.8
Hay (T4)	93.5	8.7	91.3	4.8	81.4	60.0	17.3

ADF = acid detergent fiber; ADL =acid-detergent lignin; CP =crude protein; DM = dry matter; FSL= F. sycomorus Leaf; NDF= neutral detergent fiber; OM =organic matter; FSF=F. sycomorus Fruit.

Among supplemented group, sheep in T2 had higher ($p<0.05$) dry matter intake from the basal diet followed by sheep in T3 and T4. In spite of the fact that supplemented group received equal quantity of the supplements (300 g/day), the lower basal DM intake recorded for sheep in T4 followed by T3 as compared to sheep in T2 might be explained by differences in nitrogen content of the different supplements. The total DM intake was higher ($P<0.001$) in the order T2>T3>T4 which could be attributed to differences in crude protein composition of the different types of supplements.

Table 2. Daily dry mater intake of Washera sheep fed natural pasture hay alone and supplemented with F.sycomorus leaf, fruit and their mixtures

DM intake (g/day)	Treatments				SEM	Pr>F
	T_1	T_2	T_3	T_4		
• Basal	581.7^{a}	429.9^{b}	402.6^{c}	375.4^{d}	3.85	<.0001
• Supplement	-	300.0	300.0	300.0	-	-
• Total DMI	581.7^{d}	729.9^{a}	702.8^{b}	675.4^{c}	13.18	<.0001
DMI as %BW (%)	3.0^{b}	3.7^{a}	3.6^{a}	3.5^{a}	0.07	0.0387
DMI as MBW(g/kg$^{0.75}$) Kg$^{0.75}$(BW$^{0.75}$)	66.0^{d}	73.4^{a}	71.9^{b}	69.5^{c}	0.36	0.001
Total CPI	45.7^{d}	85.8^{a}	79.2^{b}	72.7^{c}	3.51	<.0001
Total OMI	538.7^{d}	662.7^{a}	646.8^{b}	631.3^{c}	11.19	<.0001
Total NDFI	360.1^{d}	484.8^{a}	422.4^{b}	393.9^{c}	2. 58	<.0001
Total ADFI	303.2^{d}	392.0^{a}	347.6^{b}	317.5^{c}	7.87	<.0001
Total ADLI	101.5^{d}	122.2^{a}	113.3^{b}	104.5^{c}	1.89	<.0001
ME (MJ /kg DM)	4.9^{c}	8.0^{a}	7.7^{ab}	7.4^{b}	0.29	<.0001
Substitution rate	-	0.51^{c}	0.60^{b}	0.69^{a}	0.016	<.001

$^{a-d}$means with different superscripts in row are significantly different; ADF=Acid detergent fiber; ADL= Acid detergent lignin; CP = crude protein; DM= dry matter; ME = metabolisable energy; FSL = F. sycomorus Leaf; NDF = neutral detergent fiber; OM = organic matter; SEM= standard error of mean; FSF = F. sycomorus Fruit ; T_1= natural pasture hay alone; T_2 = hay +300 g FSL DM; T_3 = hay +300 g 1FSL:1FSF DM mix; T_4 = hay +300 g FSF on DM basis.

The total average daily CP intake was significantly lower ($P<0.001$) in un-supplemented group than supplemented sheep. This could be attributed to the relatively low CP content of the basal feed. The CP, OM, NDF and ADF intakes in the current study were significantly higher ($P<0.001$) for sheep in the supplemented group (T_2-T_4) than in the un-supplemented (T_1). This could be due to improved rumen condition created by the supplementation that enhanced feed intake. There was also significantly higher ($P<0.001$) estimated metabolizable energy intake (EME**)** for supplemented group as compared to sheep in the un-supplemented. The total DM intake as a percent of body weight was also higher for sheep in supplemented group as compared to the un-supplemented.

Among the supplements, sheep in T_2 had higher DMI expressed as percent of body weight followed by sheep in T_3 and T_4. The rate of substitution was higher in the present experiment and the difference among dietary treatments is significant (P<0.001).

Trends in total dry matter intake of Washera sheep fed natural pasture hay alone and supplemented with F. sycomorus leaf, fruit and their mixtures is presented in Figure 1.

DM = dry matter; FSL = F. sycomorus Leaf; FSF= F. sycomorus Fruit; T_1 (un-supplemented) = natural pasture hay; T_2 = hay +300g FSL; T_3 = hay + 300 g 1FSL:1FSF DM mix; T_4 = hay +300 g FSF DM.

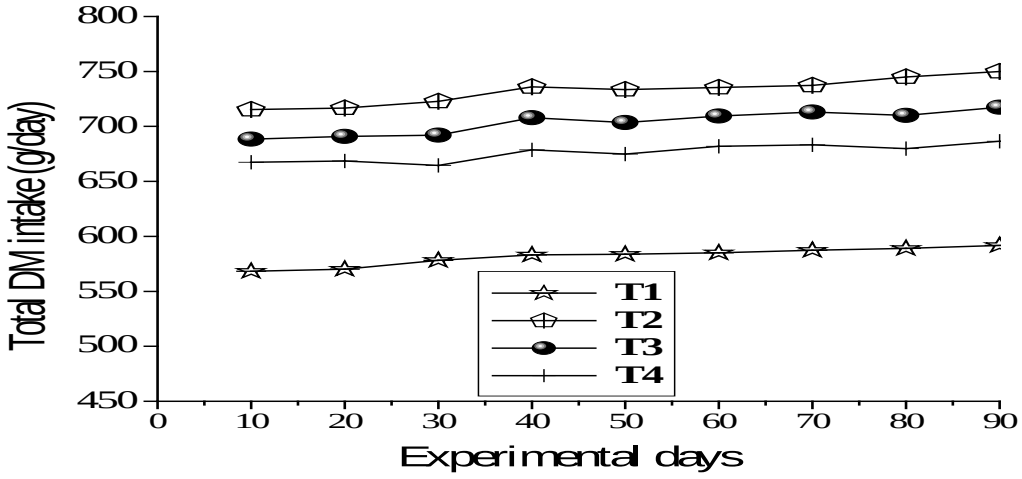

Figure 1. Trends in dry matter intake across the experimental period for Washera sheep fed natural pasture hay alone and supplemented with F. sycomorus leaf, fruit and their mixtures.

It is apparent from the figure that total feed dry matter intake increased as feeding period advanced. However, sheep in the un-supplemented group maintained lower feeding intake throughout the study period compared to supplemented animals.

Body Weight Change and Feed Conversion Efficiency

Mean initial and final body weight (FBW), average daily body weight gain (ADG) and feed conversion efficiency (FCE) of Washera sheep fed on grass hay and supplemented with F. sycomorus leaf, fruit and their mixtures are presented in Table 3 below. Supplementation significantly improved (P<0.001) daily BW gain compared to the un-supplemented. However, among the supplemented group, sheep in T2 performed significantly better (P<0.05) than sheep in T3 and T4. Supplementation also significantly increased (P<0.001) FCE and FBW of sheep compared to the un-supplemented treatment.

The lower FCE for T1 was probably because of the relatively low CP and energy intake and higher fiber content of the basal diet that might have caused the use of metabolizable energy to be depressed slightly.

Table 3. Body weight parameters, feed conversion efficiency of Washera sheep fed natural pasture hay alone and supplemented with F. sycomorus leaf, fruit and their mixtures

Parameters	T_1	T_2	T_3	T_4	SEM	Pr>F
Initial body weight (kg)	17.5	17.5	17.6	17.5	0.08	0.5888
Average daily gain (g/day)	8.0^{c}	45.1^{a}	36.9^{b}	36.2^{b}	3.41	0.0053
Final body weight (kg)	18.2^{c}	21.6^{a}	20.9^{b}	20.8^{b}	0.32	0.0058
Body weight change (kg)	0.72^{c}	4.1^{a}	3.3^{b}	3.3^{b}	0.31	<.0001
FCE (g ADG/g DMI)	0.01^{d}	0.06^{a}	0.05^{b}	0.05^{c}	0.004	<.0001

[a-c] Means with different superscripts in the same row differ significantly; DMI = dry matter intake; FCE = feed conversion efficiency; HCW = hot carcass weight; FSL = F. sycomorus Leaf; SEM = standard error of mean; FSF = F. sycomorus Fruit; T_1 = natural pasture hay; T_2 = hay + 300 g FSL DM; T_3 = hay + 300 g 1FSL:1FSF DM mix; T_4 = hay + 300 g FSF DM.

Trends in body weight change of Washera sheep fed natural pasture hay alone or supplemented with F. sycomorus leaf, fruit or their mixtures is presented in Figure 2.
FSL= F. sycomorus Leaf; FSF= F. sycomorus Fruit; T1=natural pasture hay; T2 = hay + 300g 1FSL DM; T3 = hay + 300 g 1FSL:1 FSF DM mix; T4 = hay +300g FSF DM.

It was made clear from the figure that as the feeding period advanced, body weight change of experimental animal varied. Thus, animals in the un-supplemented maintained their body weight. However, animals in the supplemented group (T_2-T_4) showed an increasing trend across the feeding period.

The relationship between average daily gain (dependent variable) and CP intake (independent variable) was assessed using simple linear regression analysis as presented in Figure 2. Thus, the fitted regression model explained 95% of the total variation (R^2=0.951). The result showed that for each unit change in CPI, ADG changes by 0.903.

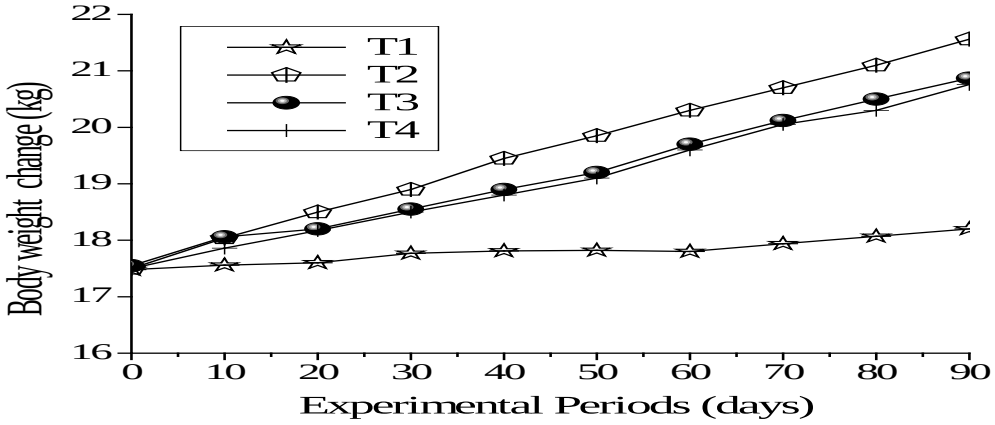

Figure 2. Trends in body weight change across the feeding period for Washera sheep fed natural pasture hay alone and natural hay supplemented with F. sycomorus leaf, fruit and their mixtures.

Partial Budget Analysis

Partial budget analysis was conducted to assess the economic benefit of supplementation with F. sycomorus leaf, fruit and their mixture feed to Washera sheep under stall feeding system Table 8 below. According to partial budget analysis, sheep supplemented with dried leaf of F. sycomorus (T_2) followed by the mixtures of its leaf and fruit (T_3) returned a higher profit margin than sheep supplemented with fruits of F. sycomorus (T_4) and the un-supplemented (T_1).

The present result suggested that supplemented with F. sycomorus leaf (T_2) and its mixture with fruit (T_3) is potentially more profitable than supplemented with fruit of F. sycomorus (T_4) or the un-supplemented (T_1). The finding was attributed to the higher ADG and final body weight recorded for sheep in T_2 and T_3. Sheep supplemented with T_3 had statistically equivalent body weight gain compared to sheep supplemented with T_4.

However, it had higher estimated selling price but lesser net income and very low change in net income. Therefore, the net return of T_2 (198.2ETB) was higher than T_3 (137.5ETB) and T_4 (127.7ETB). The difference in the net return among treatments could be attributed mainly to feed conversion efficiency.

Table 4. Partial budget analysis of Washera sheep fed natural pasture hay alone and supplemented with F. sycomorus leaf, fruit and their mixtures

Variables	Treatments			
	T_1	T_2	T_3	T_4
Purchasing price of sheep (ETB/head)	212.0	212.0	212.0	212.0
Estimated selling price of sheep (ETB/head)	265.0	440.0	380.0	375.5
Total hay consumed (kg/head)	59.0	45.5	40.3	36.9
Total leaf of F. sycomorus consumed (kg/head)	-	28.9	0.0	0.0
Total Fruit of F. sycomorus consumed (kg/head)	-	0.0	0.0	28.6
Total Fruit and leaf of F. sycomorus consumed (kg/head)	0.0	0.0	28.2	0.0
Cost for hay (ETB/sheep)	23.6	18.2	16.1	17.2
Cost for leaf of F. sycomorus (ETB/sheep)	0.0	11.6	0.0	0.0
Cost for fruit of F. sycomorus (ETB/sheep)	0.0	0.0	0.0	18.6
Cost for fruit and leaf of F. sycomorus (ETB/sheep)	0.0	0.0	14.1	0.0
Total feed cost /VC (ETB)	23.6	29.8	30.5	35.8
TRR	53.0	228.0	168.0	163.5
NI(ETB)	39.4	198.2	137.5	127.7
ΔN	0.0	145.2	-60.7	-9.8
ΔTVC	0.0	6.2	0.7	5.3
MRR	0.0	23.4	8.7	1.9

▲NI= change in net income; ETB= Ethiopian Birr; FSL=F. sycomorus Leaf MRR= marginal rate of return; NI= Net income; Suppl. = supplement; FSF= F. sycomorus Fruit; TR= total return; TVC= total variable cost; T_1= natural pasture hay; T_2= T_1+ 300g leaf; T_3= T_1+150g leaf: 150g fruit mix; T_4= T_1 + 300 g fruit. NB=Feed stuff Cost used to conduct the experiment were natural pasture hay, F.sycomorus leaf and F.sycomorus fruit 40 ETB /Qt,40 ETB / Qt, 70 ETB/Qt respectively and .the partial budget of the experiment was done by considering the labor cost as the price of F. sycomorus tree leaf and fruit. ETB = Ethiopian birr Qt = Quintile

The present result suggested that supplemented with F. sycomorus leaf (T_2) and its mixture with fruit (T_3) is potentially more profitable than supplemented with fruit of F. sycomorus (T_4) or the un-supplemented (T_1). The finding was attributed to the higher ADG and final body weight recorded for sheep in T_2 and T_3. Sheep supplemented with T_3 had statistically equivalent body weight gain compared to sheep supplemented with T_4. However, it had higher estimated selling price but lesser net income and very low change in net income. Therefore, the net return of T_2 (198.2ETB) was higher than T_3 (137.5ETB) and T_4 (127.7ETB). The difference in the net return among treatments could be attributed mainly to feed conversion efficiency.

DISCUSSIONS

Chemical Composition of the Experimental Feeds

The chemical composition of feedstuff used in this study is given in Table 1 below. In the current experiment the CP content of F. sycomorus leaf was 17.9%. Similar result (17.95%) was also reported by Nkafamiya *et al.* (2010). On other hand, Njidda and Ikhimioya (2010) and Lorenzo (2002) reported 14.9 and 22.1% CP for F. sycomorus leaf, respectively. The NDF, ADF, ADL, DM and ash content of F. sycomorus leaf on DM basis in this study was 64.6%, 52.5%, 17.4%, 93.2% and 11.9%, respectively. The results for NDF and ADF composition were higher than 54.8% and 33.4% reported by Njidda and Ikhimioya (2010). However, literature is inconsistent with regard to the CP composition of F. sycomorus leaf. Nkafamiya *et al.* (2010) reported 17.95%. The DM content of F. sycomorus leaf documented in this study was lower than 95.6% reported by Njidda and Ikhimioya (2010) but higher than 85.9% reported by Nkafamiya *et al.* (2010). The ash content of F. sycomorus leaf in the current study is higher than 9.5% reported by Nkafamiya *et al.* (2010) but lower than 18% reported by Njidda and Ikhimioya (2010). Moreover, the ash composition of the leaf of F. sycomorus was higher than fruit of the same plant and natural pasture hay.

On the other hand, the CP content of the F. sycomorus fruit in the current study was higher than 6.9-9.5% reported by Makishima (2005) and Lorenzo (2002). The difference in

chemical constituents between the present findings and literature might be attributed to differences in harvesting season, stage of harvesting, growth pattern of the species within the genus, genetic potential, bioclimatic conditions and cropping systems as reported by Chanda and Bhaid (1987) and Divakaran *et al.* (1985).

The hay offered to the experimental animals in the current study had CP content of 7.9% with higher NDF and ADF composition. Even though the CP content of hay recorded in the present study was higher than 4.2% and 3.7% reported by Mulu *et al.* (2008) and Asnakew (2005), respectively; and lower than 10.9% reported by Yihalem (2004), it was expected to meet maintenance requirement of the animals. Therefore, the hay used in the current study was characterized as good quality hay in terms of CP content. It has been stated that CP value ranging from 7-7.5% is required to satisfy the maintenance need of ruminants (Van Soest, 1982).

Feed Intake

The basal feed DM intake was higher ($P>0.05$) for sheep fed on T_1 diet as compared to sheep in supplemented group (T_2-T_4). Among supplemented group, sheep in T_2 had higher ($p<0.05$) dry matter intake from the basal diet followed by sheep in T_3 and T_4. In spite of the fact that supplemented group received equal quantity of the supplements (300 g/day), the lower basal DM intake recorded for sheep in T_4 followed by T_3 as compared to sheep in T_2 might be explained by differences in nitrogen content of the different supplements. Consequently, this had negatively impacted total dry matter intake. Topps (1997) reported that supplementation level beyond 30-40% of the total DM offered reduces intake of the basal feed.

The total DM intake was higher ($P<0.001$) in the order $T_2>T_3>T_4$ which could be attributed to differences in crude protein composition of the different types of supplements. The supplemented sheep consumed higher total DM because supplementation might have created a favorable rumen environment resulting in enhanced fermentation of the basal roughage and thus increased microbial protein synthesis (Osuji *et al.*, 1995). The positive effects of supplementation on feed intake may have been a reflection of the increase in the intake of essential nutrients such as energy, vitamins and minerals and in particular nitrogen. Moreover, the high total DM intake in supplemented group could be due to lower gut fill of the supplements compared to natural pasture hay. The increase in total DM intake due to supplementation in the present study was in agreement with the result reported by Hirut (2008) and Wondwosen (2008).The total average daily CP intake was significantly lower ($P<0.001$) in un-supplemented group than supplemented sheep. This could be attributed to the relatively low CP content of the basal feed. However, the CP content of the basal diet was slightly higher than maintenance requirement of small ruminants (Minson, 1990; Gatenby, 2002).

The CP, OM, NDF and ADF intakes in the current study were significantly higher ($P<0.001$) for sheep in the supplemented group (T_2-T_4) than in the un-supplemented (T_1). This could be due to improved rumen condition created by the supplementation that enhanced feed intake. Adugna and Sundstol (2000) also reported that the increased intake in the supplemented group could be due to increased availability of nitrogen to rumen microbes and enhanced rate of digestion. Among supplemented group, however, it was higher for sheep in T_2 followed by T_3 and T_4. The higher CP contained in F. sycomorus leaf (17.9%) as compared to F. sycomorus fruit (11.8%) might have rendered sheep in T_2 followed by T_3 to have higher intakes. According to Kempton *et al.* (1979) and Dothi (2001), dietary protein supplementation is known to improve intake by increasing the supply of N to the rumen microbes or can be increased by reducing poor quality feed retention time after supplementing concentrates to micro-organisms and stimulating their function in the rumen. There was also significantly higher ($P<0.001$) estimated metabolizable energy intake (EME**)**

for supplemented group as compared to sheep in the un-supplemented. The intake of energy increased in the order of $T_2 > T_3 > T_4$ with increased level of F. sycomorus leaf as suggested previously (Montaldo, 1972).

The total DM intake as a percent of body weight was also higher for sheep in supplemented group as compared to the un-supplemented. Among the supplements, sheep in T_2 had higher DMI expressed as percent of body weight followed by sheep in T_3 and T_4. The results obtained in the current study were not very different from those reported for various breeds of sheep and goats in the tropics (Devendra and Burns, 1983).

The rate of substitution was higher in the present experiment and the difference among dietary treatments is significant ($P<0.001$). Substitution rates are often low when animals consume forage of low to medium digestibility. Doyle *et al.* (1988) suggested that the rate at which basal hay intake reduce with increasing supplement intake (the substitution rate) reflects directly the effect of the supplement on the fractional rates of digestion and outflow from the rumen. Those supplement feeds with rapid fermentation rate replace the basal roughage to a lower extent than those that ferment slowly (Nsahalai and Ummuna, 1996).

Body Weight Change and Feed Conversion Efficiency

Supplementation significantly improved ($P<0.001$) daily BW gain compared to the un-supplemented. However, among the supplemented group, sheep in T2 performed significantly better ($P<0.05$) than sheep in T3 and T4. Supplementation also significantly increased ($P<0.001$) FCE and FBW of sheep compared to the un-supplemented treatment. The lower FCE for T1 was probably because of the relatively low CP and energy intake and higher fiber content of the basal diet that might have caused the use of metabolizable energy to be depressed slightly. Adebowale *et al.* (1991) also reported that the low degree of digestion coupled with low passage rate through the alimentary tract limit net energy availability for production. However, supplemented sheep (T2-T4) did significantly ($P>0.01$) differ in these parameters.

Supplementation of MPT to small ruminants improved growth performance as documented earlier (Melaku *et al.*, 2004; Reed *et al.*, 1990). Similarly, Aynalem and Taye (2008) reported average daily gain of 43.3, 50.5 and 95.1 g/day in lambs supplemented with 200, 300 and 400 g/day Girawa, respectively. It has been reported that fodder trees would be good protein supplements for ruminants, provided that they are degraded adequately in the rumen to make the protein available to the animal and non-toxic (Leng, 1997). Anugwa and Okori (1987) reported that, West African dwarf lambs gained 71 g/day over a 14-day period when fed a sole diet of F. elasticoides foliage. However, the F. sycomorus leaf, fruit and their mixture in the current study could change the body weight gain, possibly sufficient supply of protein.

Generally, supplementation with MPTs like F. sycomorus leaf, fruit and their mixture appeared to improve daily BW gain of sheep, probably either by providing nutrient available for absorption or by enhancing microbial protein synthesis. Though there has not been exhaustive study conducted on F. sycomorus in Ethiopia on one hand and Washera sheep on the other, the average daily gain recorded in the present study was small compared to literature. This might be attributed to the alkaloid concentration of F. sycomorus and/or size of the sheep breed used for the study. Similarly, in this study, sheep fed natural pasture hay alone exhibited mean BW gain of 8 g/day. This positive ADG indicates that natural pasture hay used in the current experiment provided nutrients sufficient for maintenance requirements of the animals.

CONCLUSION

This experiment verified that supplementing intact male yearling Washera sheep with natural pasture hay alone (T_1) or natural pasture hay supplemented with either leaf (T_2), mixture of leaf and fruit (at a ratio of 1:1; T_3) or fruit (T_4) of F .sycomorus improved average daily body weight gain, empty body weight and hot carcass weight supplemented compared with un-supplemented (T1). In general, supplementation with 300g FSL, FSF and their mixture (FSL: FSL) improved the performance of sheep compared to the un-supplemented. Among the feeding strategy employed, supplementing sheep with T_2 becomes biologically optimum and economically feasible.

RECOMMENDATIONS

Due to shortage of pasture/grazing land, high cost of agro industrial by products and increasing competition with other livestock, it is economical and biologically advantageous to use F. sycomorus leaf and its fruits as supplement to improve productivity of sheep. Since supplementation with F. sycomorus leaf resulted in the highest performance parameters in sheep and returned the highest income, small holder farmers are advised to supplement sheep with leaf in order to exploit the genetic potential of the animals.

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Original Article

Study of Toll-Like Receptor 9 Gene Polymorphism and its Association with Mastitis Disease in the Holstein Cows

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ABSTRACT

Mastitis is causes considerable economic losses due to decrease in the quality and quantity of milk production, increases of the cost of treatment and veterinary services, and animal waste (increases of waste product). Inflammation of the udder caused by a traumatic event, toxic agent, or invasion of microorganisms may be indicated by an increase in numbers of somatic cells in milk. Bovine TLR9 is located on BTA22. The TLR9 mRNA consists of two exons and is 3255 bp including 5′ and 3′ UTRs, The genomic size of TLR9 is 4264 bp and the protein is 1029 aa. Blood samples were collected from 150 dairy cattle from six herds. DNA extraction was performed by salting out method. A fragment of 245 bp from intron 1 was amplified by the polymerase chain reaction and analyzed by single-strand conformation polymorphism to get the patterns of single-stranded DNA separated by native polyacrylamide gel electrophoresis and visualized by silver staining. Nine genotypes were revealed with the frequencies of 0.8150 (AA), 0.1481 (AB), 0.2666 (AC), 0.1704 (AD), 0.8880 (BB), 0.1480 (BC), 0.5920 (BD), 0.2960 (CC) and 0.1407 (CD). The allele frequencies for A, B, C and D were 0.3741, 0.2000, 0.2407 and 0.1852; respectively.Chi-square test didn't confirm Hardy-Weinberg (H-W) equilibrium for this locus. Associations between polymorphisms and the trait studied were evaluated using the MIXED procedure of the SAS 9.1 software. Results showed that the somatic cell score not have a significant association with genotypes of TLR9 gene.

Keywords: Holstein cow, polymorphism, TLR9 gene, somatic cell score.

INTRODUCTION

Bovine mastitis

Bovine mastitis is a disease with high incidence worldwide, even in herds with mastitis control programs. It causes considerable economic losses due to decreases in the quality and

quantity of milk production, increases in the cost of treatment and veterinary services, and animal waste (Crist *et al*., 1997; Dego *et al*., 2002).There are many different contagious and environmental bacteria that cause mastitis (Hogan and Smith., 1998).Inflammation of the udder caused by a traumatic event, toxic agent, or invasion of microorganisms may be indicated by an increase in numbers of somatic cells in milk. Although SCC from an uninfected quarter average about100 x 10^3 cells/ml, the *SCC* in infected mammary quarters is generally much higher (Mattila, 1985; Sheldrake *et al*., 1983 a,b).
The conserved molecules that are unique to some classes of potential pathogens known as pathogen associated molecular patterns (PAMPs) are primarily identified by an array of specialized pattern recognition receptors (PRR) of innate immune system, which includes the TLR family (Toll-like receptors) (Medzhitov *et al*., 1997; Akira, 2001; Janeway and Medzhitov, 2002).

Molecular structure of TLRs

TLRs are evolutionarily conserved innate immune receptors that belong to a family of type Itransmembrane proteins with an extracellular amino terminus. The ectodomain of TLR molecules consists of 16-28 leucine rich repeat (LRR) domain (Matsushima *et al*., 2007), which is mandatory for identification of PAMPs (Fujita *et al*., 2003). The central part of the LRRs possesses more irregular or longer motifs and varies among different TLRs, implying the functional importance of the central parts in ligand recognition (Matsushima *et al*., 2007). The cytoplasmic regions of TLR molecules possess a conserved domain Toll/IL-1 receptor (TIR) domain involved in downstream signal transduction. Based on their localization in the cell and the ligands they recognize, TLR molecules are divided into two groups. TLRs of the first group (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10) are expressed on cell surface and recognize compounds derived mainly from microbes. TLRs belonging to the second group (TLR3, TLR7 and TLR9) are expressed on the membranes of intracellular organelles such as endosomes (Heil *et al*., 2003; Matsumoto *et al*., 2003) and recognize nucleic acids or derivatives of nucleotides.

TLR9 structure

TLR9 has been grouped in a subfamily with TLR7 and TLR8, receptors that are also expressed within the endosome and sense pathogen-derived RNA and DNA. ClustalW multiple sequence alignments (www.ebi.ac.uk/clustalw) of these mammalian TLR9 sequences revealed that the bovine TLR9 shared 79% homology with human TLR9 and 73% homology with murine TLR9.Bovine TLR9 is located on BTA22. The TLR9 mRNA consists of two exons and is 3255 bp including 5′ and 3′ UTRs, according to the NCBI reference sequence (Accession No. NC_007320). The genomic size of TLR9 is 4264 bp and the protein is 1029 aa (Cargill and Womack, 2007).

Cellular responses to TLR9 signaling

A variety of cells have been shown to respond to CpG ODN stimulation but these responses may reflect either direct stimulation through TLR9 or indirect activation through CpG ODN-induced cytokine secretion. In humans, it appears that only B cells and plasmacytoid dendritic cell (pDC) express TLR9 and respond directly to CpG ODN stimulation (Hornung *et al*., 2002; Bauer *et al*., 2001). The production of IFN-a by pDC plays a key role in the activation of NK cells and other innate immune responses. A similar cellular pattern of TLR9 expression has been reported for mice with the exception that myeloid DC and macrophages also respond directly to CpG ODN stimulation (Sparwasser *et al*., 1997, 1998). There is evidence that CpG ODN can directly stimulate purified bovine B cells to proliferate and express IL-6 and stimulate purified bovine monocytes and macrophages to express IL-6 and IL-12 (Brown *et al*., 1998; Zhang *et al*., 2001). Cultured bovine macrophages and myeloid DC (derived from CD14+ monocytes) were also used to study CpG

ODN-induced responses and CpG-specific induction of IL-10, IL-12 and TNF secretion was observed (Werling *et al*., 2004). To date, there have no study which has been showed association between TLR9 and mastitis disease.
The objectives of the current study were to detect polymorphisms of TLR9 and determine association of such polymorphisms with Somatic Cell Score in Holstein cattle of RazaviKhorasan province.

MATERIAL AND METHODS

Blood samples collection and DNA processing

Blood samples (at 10 ml volume, the tubes containing of Na_2EDTA) of 150 Iranian Holstein cows (female, upper than 2 lactation period) were collected randomly from six Iranian Holstein cattle farms in RazaviKhorasan. The blood samples were kept to isolated in -20 °C. Genomic DNA was isolated from blood samples by the salting out method (Iranpur and Esmailzadeh, 2010) and the quality and quantity of DNA was investigated by Nanodrop set and loaded on a 1% agarose gel. Polymerase chain reactions (PCR) were carried out in 25 µl volume including 250 ng of genomic DNA, 10 pmol each primer (Figure1) and 12µl Master Mix (sinaclone company, Iran).

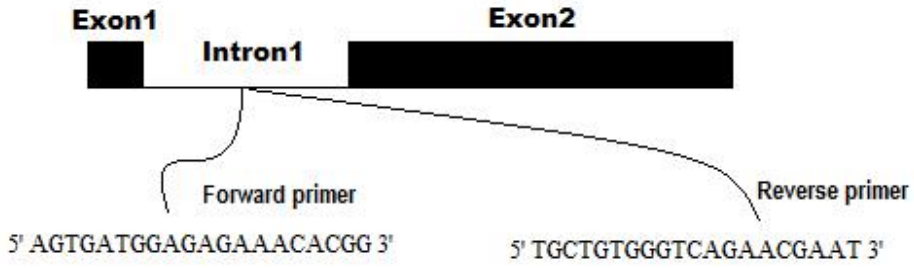

Figure 1: The selection subregion of primers and their sequencing

The PCR protocol was 95 °C for 5 min, followed by 35 cycles of 95° C for 30 s, annealing for 40 s and 56°C for 40 s, 72 °C for 45s with a final extension at 72°C for10 min. 1% agarose gel and Marker (500bp, Fermentaz company) was used to investigation quality of the PCR products (figure 2).

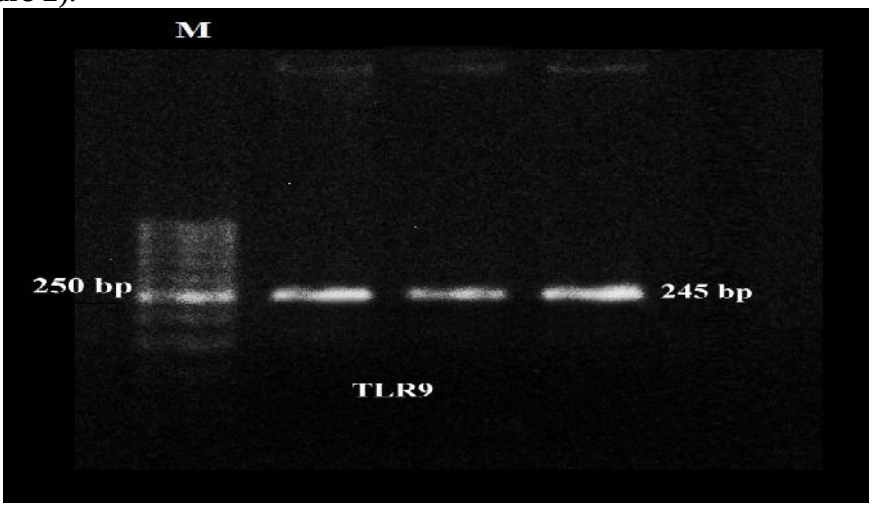

Figure 2: Quality of PCR products for TLR9 gene, identified by Marker (500bp from fermentaz Co).

Genotyping

The PCR products were genotyped by single-strand conformation polymorphism (SSCP) to screen the mutations within the amplified region. In total, 3µl of the PCR product of each sample was mixed with 7µl of denaturing buffer (98% formamide, 1% NaoH, 0.5% bromophenol blue and 0.5% glycerol) and then denatured at 95°C for 10 min, followed by a rapid chill on ice for 10min.

The denatured PCR products were electrophoresed on 10% polyacrylamide gels for 12 h at 8 V/cm and stained by 0.2% AgNO3 for 20min. Genotypes were recorded according to band patterns.

Statistical Analysis

The program POPGENE 32 (Francis *et al*., 1999) was used to test the number of alleles per locus (N), effected number of alleles (Ne), expected (He) and observed (Ho) heterozygosity, and departures from Hardy–Weinberg equilibrium (HWE).

The relationship between different genotypes and SCS trait, which was Log of Somatic Cell Count, were analyzed using mixed procedure of SAS9.1 package (SAS Inst. Inc., Cary, NC).

Tukey-Kramer test were used to compare the mean values of the attributes traits for the different genotypes. The statistical model is as follows:

$$ScSijkmno = \mu + Cowi + Gj + Hk + Ym + Sn + Dim + qDim + Po + Milkij + FPij + PPij + E_{ijkmno}$$

Where:
SCS_{ij}- somatic cell score of cow i and genotype j.
µ- means of population,
Cow_i- The fixed effect of cow i,
G_j- the fixed effect of genotype j,
H_k- the fixed effect of herd k, (k=1, 2, 3, 4, 5, 6).
Y_m- the fixed effect of calving year m.
S_n- the fixed effect of calve season n, (n=1, 2, 3, 4)
DIM- the number of lactation days.
qDIM- square of the number of lactation days.
P_o- parity O of cow, (O= 2,3,4,5,6,7)
$Milk_i$- monthly milk yields for each of cow i,
PP_i and FP_i- protein and fat percentage respectively.
E_{ijkmno}- the random errore.

RESULTS

Nine genotypes were revealed with the frequencies of 0.8150 (AA), 0.1481 (AB), 0.2666 (AC), 0.1704 (AD), 0.8880 (BB), 0.1480 (BC), 0.5920 (BD), 0.2960 (CC) and 0.1407 (CD). The allele frequencies for A, B, C and D were 0.3741, 0.2000, 0.2407and 0.1852, respectively.The chi-square test confirmed that this population is not in H-W equilibrium for the studied locus (Figure3 and Table1).

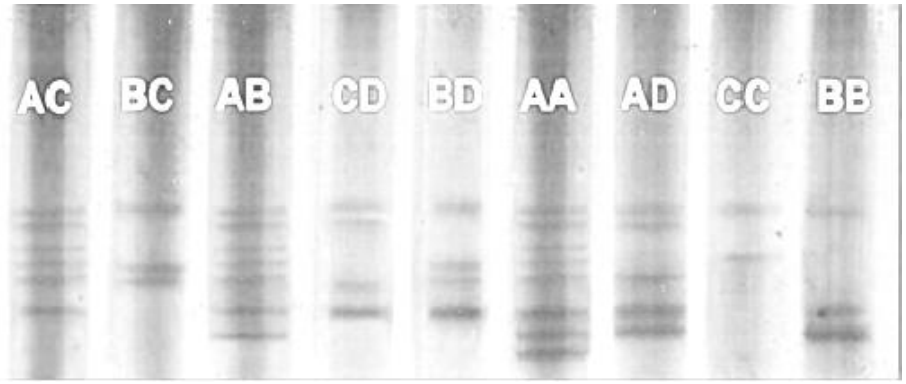

Figure3: genotypes derived from the electrophoresis of Polyacrylamide gel for TLR9 gene.

Table 1: the frequencies of Allele and genotypes for TLR9 gene

Allele frequency (%)				Genotype frequency (%)						
A	B	C	D	AA	AB	AC	AD	BB	BC	X^2
37.41	20.00	24.07	18.52	08.15	14.81	26.66	17.04	08.88	01.48	38.15
				BD	CC	CD				
				05.92	02.96	14.07				

X^2 =chi-square for HW equilibrium

Genetic diversity parameters such as observed and expected homozygosity and heterozygosity, Observed number of alleles (Na), Effective number of alleles (Ne), Shannon and Nei index, obtained from POPGENE are shown in table 2.

Table 2: Genetic diversity values for TLR9 gene

Parameters of genetic diversity	TLR9 gene
Na	4
Ne	3.6740
Obs_Het	0.8000
Obs_Hom	0.2000
Exp_Het	0.7305
Exp_Hom	0.2695
Nei	0.7278
I	1.3448

Na = Observed number of alleles,Ne = Effective number of alleles (Hartl and Clark, 1989), I = Shannon's Information index (Shannon, 1948), Obs-Het= observatedheterozygosity, Obe-Hom= observatedhomozygosity, Exp-Het=Expected heterozygosity, Exp-Hom= Expected homozygosityand were computed using Levene (1949), Nei=Nei's index (1973).

Another criteria is used for loss of heterozygosity in the population is the fixation index that is referred to as the inbreeding coefficient (Wright, 1977). If the fixation index value is negative, indicating that the loci alleles have low correlation with each other and is high the heterozygosity in it locus, values of the fixation index was showed in table 3.

Table 3: The fixation index for TLR9 gene

Allele	Value[1]
A	-0.2496
B	0.3056
C	-0.1550
D	-0.2273
Total	-0.0992

[1] Values of negative, indicating that is heterozygosity in it locus

Association between TLR9 gene and SCS trait

The somatic cell score not showed a significant association with genotypes of TLR9 gene (Table 4).According to table 5, BD genotype have a lower SCS than other genotypes. Maybe, can be said that this genotype were considerable to breeding programs.

Table4: Statistical values of the somatic cell score trait and its association with TLR9 gene

Trait	TLR9 gene		
	Mean	RMSE	P value
SCS[1]	2.1428	0.5273	0.0955

SCS= Somatic Cell Score; RMSE= root mean square error

[1] The somatic cell score not showed a significant association with genotypes P>0.05.

Table5: Least Squares Means of genotypes for SCS trait

Trait	Genotypes (μ ± s.e.)								
	AA	AB	AC	AD	BB	BC	BD	CC	CD
SCS	2.10±0.063	2.15± 0.050	2.08± 0.046	2.19± 0.045	2.09± 0.0612	2.05±0.104	2.03±0.064	2.04±0.089	2.05±0.055

SCS= somatic cell score

DISCUSSION

Polymorphism was observed in 245bp fragment from intron 1 of TLR9 gene in Holstein cattle of RazaviKhorasan. Mastitis is the most frequent and costly disease in dairy production and the innate immune system is considered to be important as the first line defense against this disease. Mastitis infections have been correlated with over expression of TLR2 and TLR4 in mammary glands of cattle and TLR2 in pigs; this correlation is even more evident with increased severity of infection (Goldammer *et al*., 2004; Ibeagha-Awemu *et al*., 2008; Petzl *et al*., 2008; De Schepper *et al*., 2008; Yang *et al*., 2008a,b; Zhu *et al*., 2008). The limited role of TLR9 in pathogen recognition of mammary gland infection of cattle have been suggested both by *in vivo* and *ex vivo* studies (Goldammer *et al*., 2004; Mount *et al*., 2009). However, species difference in TLR9 expression during mastitis exists as CpG-ODN has been shown to promote the expression of its specific receptor (TLR9 mRNA) in goat mammary tissue (Zhu *et al*., 2007). In cattle significant association exists between TLR2 and TLR4 polymorphisms and mastitis indicated by somatic cell scores, an indirect index to measure the mastitis phenotype (Wang *et al*., 2007; Pant *et al*., 2008; Zhang *et al*., 2009). In contrast, Opsal *et al*. (2008) failed to detect any significant association between the chromosomal regions surrounding TLR2 and TLR4 and mastitis in Norwegian red cattle. TLR genes 2, 4 and 6 polymorphisms and their relationship with somatic cell count and natural bacterial infections of the mammary gland in sheep have also been reported (Swiderek *et al*., 2006). Moreover, TLR4 polymorphisms in cattle have also been shown to be associated with lactation persistency (Sharma *et al*., 2006). Based on established association between TLRs and somatic cell scores, selecting animals for breeding programs with specific TLR gene polymorphisms could help improve herd resistance to mastitis. However, the somatic cell score not showed a significant association with TLR9 gene polymorphisms.

CONCLUSION

Probability, one of the reasons that there was no significant association between genotypes and SCS trait, can be small sample size and large genotypic variation in this research.

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Original Article

Dairy Cattle Production System in Central Zone of Tigray: in The Case of Aksum and Adwa

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ABSTRACT

The purpose of this study was to explore the management practices of dairy cattle production in Central Zone of Tigray. A total of 160 dairy cattle holding households were selected by systematic random sampling technique. The study employed multiple methods of data analysis including descriptive statistics, Chi-square test, T-test and qualitative analysis. Majority of the urban dairy farmers depend on hay, crop residues and concentrates for feeding their dairy cattle. Whereas, the peri-urban dairy farmers rely on dry and green roughages but less on concentrate feeds. In the urban areas, pipe water was the most common water source for the dairy cattle whereas, in the peri-urban areas, the use of river was very high. Significantly ($P<0.05$) better watering frequency was observed in urban than peri-urban areas. Access to veterinary services was significantly ($P<0.05$) better in urban areas than the peri-urban areas. About 44% of the peri-urban dairy farmers trek their dairy cattle more than 6km in searching veterinary services. Waste management was relatively problematic in urban areas (36.25%) as compared to the peri-urban areas (13.75%). There is also promising urban-peri-urban linkage in dairy product and by-product supply. Therefore, an appropriate feeding, watering, health management, housing and manure utilization could be the management options to improve the existing problems.

Keywords: Farm size**,** high grade exotic breed, manure utilization.

INTRODUCTION

The urban and peri-urban dairy cattle production has been developed in response to the fast growing demand for milk and milk products. Many countries have experienced very vast development in dairy sector in or around the largest urban centers, responding immediately to the market demand and profiting from the lack of links between the rural producer and the urban consumer. In Ethiopia too, urban and peri-urban dairy production systems are emerging as an important component of the milk production system. This system is contributing

immensely towards filling in the large demand-supply gap for milk and milk products in urban centers, where consumption of milk and milk products is remarkably high (Azage and Alemu, 1998). However, little research efforts have been made in Ethiopia in general and in central Tigray in particular on urban and peri-urban areas as most of the efforts are directed towards rural agricultural activities.

The few studies made so far are concentrated in and around Addis Ababa, capital city of the country. This has led to overlooking urban and peri-urban dairying from incorporating to the country's research agenda and to the overall agricultural development program. This picture has to be changed as urban dairying is becoming an important agricultural activity around major urban and peri-urban centers far from Addis Ababa. Hence, in order to design relevant dairy development strategies and implement context specific interventions for future development of the urban and peri-urban dairy production, characterization of the management practices of dairying are important. The scientific information regarding the aforementioned parameters helps as a starting point for further development endeavors in dairy enterprise in the nation. Therefore, the objective of this study is to identify the existing management practices in respect to urban and peri- urban areas.

MATERIALS AND METHODS

The study was carried out in central zone of Tigray region, Northern Ethiopia. Central Tigray Zone is one of the five zones in Tigray National Regional State. The zone approximately extends between $13^0$15' and $14^0$39' North latitude, and 38^0 34' and $39^0$25' East longitude. The altitude of the zone mainly falls within the category of 2000 to 3000 masl. Large part of the zone receives mean annual rainfall ranging from 400 to 800mm. The mean monthly maximum and minimum temperature of the zone are 30^0c and 10^0c, respectively (NMSA, 1996). The zone has the largest human population in the region. The specific study sites were Adwa & Axum urban and peri-urban areas with 1006 & 1024 kilometers far from Addis Ababa, capital city of Ethiopia, respectively. These two districts were selected purposively based on their conducive agroecological conditions for dairy production and large human population in the zone.

A cross-sectional survey was used in order to collect data on management practices of the dairy cattle. A multi-stage sampling technique was used in the cross-sectional survey. First cattle holding households were clustered in to urban and peri-urban. Aksum and Adwa, the larger towns in the zone, were considered as urban. The smaller towns found within a radius of 20km from the centers of the two larger towns were considered as peri-urban. Finally, based on the sampling frame obtained from the district office of Agriculture, a total of 160 cattle holding households were chosen using systematic random sampling technique. Pre-tested formal questionnaire was used in the cross sectional survey. The collected data included herd composition, feed resource and feeding, water sources and frequency of watering, veterinary services, waste disposal and manure utilization. Inferential and descriptive statistics were used to analyze the data using SPSS 16.0 (SPSS, 2007) software.

RESULTS

Management Practices of Dairy Cattle

Feed resources and feeding

As depicted in table 1, the major sources of feed for cattle in the study area were hay, crop residues, grazing, crop after math and non-conventional feedstuffs (like: 'Atela', kitchen waste and weeds). Grazing was practiced by small farmers and mostly for local animals in peri-urban areas, though there was a practice to some extent in urban areas. Majority of the urban dairy producers rely on zero grazing but smaller proportions were used roadsides, hillsides and vacant plots for grazing to their dairy cattle.

Table 1. Proportion of households using different types of feeds across the different locations

Feed types	Urban (N=80)				Peri-urban (N=80)			
	Dry season		Rainy season		Dry season		Rainy season	
	N	(%)	N	(%)	N	(%)	N	(%)
Crop residue	66	82.5	71	88.75	71	88.75	74	92.25
Natural pasture	10	12.5	11	13.75	29	36.25	29	36.25
Hay	80	100	63	78.75	75	93.75	28	35
Stover	66	82.5	13	16.25	58	72.5	6	7.5
Bran	65	81.25	60	75	37	46.25	27	33.75
Balanced feed	11	13.75	10	12.5	4	5	5	6.25
Improved forage	16	20	13	16.25	27	33.75	17	21.25
Cakes	41	51.25	31	38.75	12	15	14	17.5

N=Number of respondents

The figures obtained in the present study were larger as compared to the above mentioned reports. The main cause for lower access of urban dairy farms to grazing lands might be due to a decrease in grazing land as a result of urbanization and population growth. Large quantities of crop residues, mainly stover and straw, were produced from the surrounding rural areas and were supplied to the larger towns and village towns. In dry season Stover was used by 82.5% and 72.5% of the respondents in urban and peri-urban areas, respectively, as the main crop residue to feed animals (Table 1). Teff, barley, wheat and finger millet straws were stored around home to be used during critical feed shortage periods. In the peri-urban areas, there was a clear variation in the use of hay and stover across the season. Hay and stover were consumed highly during the dry season (93.75% and 72.5%, respectively) as compared to the rainy season which was 35% and 7.5%, respectively.

Water sources and watering

A higher proportion of the respondents indicated that their major water source was pipe water followed by Borehole in the urban areas whereas, in the peri-urban areas it was pipe water followed by river both during the dry and rainy seasons (Table 2).

Table 2. Water sources in dry and rainy season among the urban and peri-urban areas

Parameters	Location				Overall	
	Urban (N=80)		Peri-urban (N=80)			
	N	%	N	%	N	%
Dry season						
Borehole	15	18.75	9	11.25	24	15
Dam/pond	-	0	4	5	4	2.5
River	3	3.75	29	36.25	32	20
Spring	-	0	2	2.5	2	1.25
Pipe water	62	77.5	36	45	98	61.25
Rain water	-	0	-	0	-	0
Rainy season						
Borehole	16	20	8	10	24	15
Dam/pond	-	0	4	5	4	2.5
River	4	5	31	38.75	35	21.875
Spring	1	1.25	1	1.25	2	1.25
Pipe water	57	71.25	36	45	93	58.125
Rain water	2	2.5	-	-	2	1.25

N=Number of respondents

The watering frequency was significantly ($P<0.05$) better in urban areas than peri-urban areas both during dry and rainy seasons (Table 3). In the urban areas 81.25% and 16.25% of the households offer water for their cattle twice per day and freely available, respectively, during dry seasons. Whereas in the peri-urban areas, 47.5% and 35% of the dairy cattle holding households water their animals twice per day and once a day, respectively, in dry

seasons. Similar to the dry season, the watering frequency during the rainy season differs significantly (P<0.05) across the urban and peri-urban areas. Major proportions of the respondents in urban area water their cattle twice a day during the rainy season whereas in the peri-urban areas they provide once per day in the rainy season.

Table 3. Watering frequency in dry and rainy season in the study area

Frequency of watering	Location				Overall		Test	
	Urban		Peri-urban					
	N	%	N	%	N	%	χ^2	P-value
Dry season								
Freely available	13	16.25	14	17.5	27	16.875	-	-
Once/day	2	2.5	28	35	30	18.75	-	-
Twice/day	65	81.25	38	47.5	103	64.375	29.65	0.000
Rainy season								
Freely available	9	11.25	9	11.25	18	11.25	-	-
Once/day	14	17.5	47	58.75	61	38.125	-	-
Twice/day	57	71.25	21	26.25	78	48.75	-	-
Once in two days	-	-	3	3.75	3	1.875	37.45	0.000

N=Number of respondents χ^2=Chi-square

Animal Health Care

The study showed that, 78.75% from the urban dwellers and 75% from the peri-urban used the local veterinary clinic to treat their cattle. About 21.25% from the peri-urban and 11.25% from the urban took their cattle to traditional healer when their cattle get ill. The reason why they fail to take their dairy cattle to the local veterinary clinic was because of ineffective services rendered (56.1%), unavailability of drugs (31.7%) and unaffordable price of drug (12.2%) in urban areas. Whereas in the peri-urban areas, 61.1%, 22.2% and 12.96% respondents explained that the price of drug was not affordable, lack of knowledge about the service and ineffective services rendered, respectively (Table 4).

Table 4. Dairy cattle health management and sanitation aspects in the study area

Parameters	Location of the farm						
	Urban(N=80)		Per-urban(N=80)		Test		
	N	%	N	%	df	χ2	p-value
How do you manage cattle health problem?							
Take to local vet. Clinic	63	78.75	60	75	1	2.03	0.155
Take to traditional healer	9	11.25	17	21.25			
Private service	8	10	3	3.75			
Do you practice deworming?	70	87.5	59	73.75	1	4.84	0.028
Do you get service you intended for in local vet. clinic?	65	81.25	59	73.75	1	0.5	0.481
Reason why they fail to take their cattle to local vet. Clinic (*, **)							
Price of drug is not affordable	5	12.2	33	61.1	3	17.5	0.013
Drugs are not available	13	31.7	2	3.7			
Services are not rendered as they should be	23	56.1	7	12.96			
Lack of knowledge about the Service	-	-	12	22.2			

N=Number of respondents, χ^2= Chi-square, *Number of respondents in urban are 41, **Number of respondents in peri-urban are 54.

Majority of the urban dairy farmers kept their dairy cattle in significantly (P<0.05) better sanitary condition than the peri-urban dairy farmers. Mastitis was the most common disease in dairy farms mostly in high yielding but less hygienic dairy farms. As depicted in table 5, prevalence of mastitis was significantly (P<0.05) higher in urban than peri-urban areas. As 33.75% and 18.75% of interviewed dairy cattle holding households faced incidence of mastitis in their farms in the urban and peri-urban areas, respectively.

Access and Distance to Veterinary Clinic

Veterinary service was given both by government and private sectors in the urban areas. Mostly, the animal health clinics were cited in the urban areas. Those clinics conduct examination, treatment and vaccination for various diseases. As presented in Table 5, 92.5% and 7.5% of the respondents in the urban areas indicated the presence of access to governmental veterinary clinic and private veterinary services, respectively, whereas all respondents from the peri-urban rely totally on governmental veterinary services.

Table 5. Access and distance of veterinary clinic in the study area

Access and distance to veterinary clinic		Location of the farm				Test	
		Urban		Per-urban			
		N	%	N	%	P-value	χ^2
Access to veterinary clinic	Governmental	74	92.5	80	100	0.044	6.23
	Private	6	7.5	-	0	-	-
Distance of governmental vet. service							
	<1km	25	31.25	17	21.25	0.000	25.2
	1-5km	52	65	36	45	-	-
	6-10km	3	3.75	17	21.25	-	-
	>10km	-	-	10	12.5	-	-
Incidence of mastitis		27	33.75	15	18.75	0.031	4.65

N=Number of respondents χ^2=Chi-square

The majority of the urban dairy producers received veterinary services at the radius of one to five kilometers. Hence, the response of respondents pertaining distance to the nearest veterinary service varied significantly (*P<0.05*) across the locations. None of the respondents from the urban trek their animals beyond ten kilometers to get veterinary services but 12.5% of the interviewed households in peri-urban areas traveled greater than ten kilometers to search veterinary services for their dairy cattle.

Waste Management

Waste disposal was significantly problematic in urban (36.25%) area as compared to peri-urban (13.75 %) areas (Table 6).

Table 6. Waste disposal and manure utilization in the study area

Variables	Location of the farm				Overall		Test	
	Urban(N=80)		Per-urban(N=80)					
	N	%	N	%	N	%	χ^2	p-value
Problem of waste disposal	29	36.25	11	13.75	40	25	10.8	0.001
Use of manure								
Source of income	8	10	15	18.75	23	14.375	-	-
Fertilizer (own farm)	17	21.25	33	41.25	50	31.25	-	-
Fuel wood	55	68.75	32	40	87	54.375	26.33	0.000

N=Number of respondents χ^2=Chi-square

As compared to the peri-urban areas, a higher proportion (68.75%) of respondents in the urban areas use manure for fuel wood purpose whereas, in the peri-urban areas, 41.25% reported manure to be used as fertilizer followed by fuel wood (40%).

DISCUSSION

Farms found in intra-towns both in Adwa and Axum had little access to grazing land. Hence, mainly depend on purchased hay and agro-industrial by-products. Hay was purchased immediately after the end of rainy season and stored in hay shed for feeding throughout the year especially in the urban areas. In line with this, Negusie (2006) justified that the reason for dependence of almost all of the urban farms on hay was attributed not only to relatively better quality of the feed and less access to other feeds like natural pasture, improved forages and other crop residues due to less land to grow but also to a coping mechanism against feed shortage through the use of conserved feed. According to Yoseph *et al*. (2000) in Addis Ababa, about 7 % intra-urban and 33 % large peri-urban farms used grazing along roadside and native pasture, respectively. Similar finding was also reported by Ike (2002) from Awasa which depicted 95%, 3.3% and 1.7% of the urban farms practiced zero grazing, both zero and semi-grazing, and semi-grazing systems, respectively.

From the information obtained during group discussion, locally prepared concentrate feeds using pulse hulls and corn were also given to animals raised especially in urban areas. There were local milling factories on which farms depend to get wheat bran. The costs of concentrates were unaffordable for the majority of the dairy farmers particularly to the peri-urban dairy farmers. Regardless of the cost, large proportion of urban farms were using concentrates since they become conscious about the advantage of using concentrate feeds for increased milk yield. This indicated the existence of massive opportunity for local retailers to step up their trade and potential for investors to set up feed processing plants. So that farmers could get the concentrate feeds near and increase the productivity of their dairy animals. Hence, the supply of dairy products to the high demand in the area could be optimized.

The study of Sentayehu *et al*. (2008) showed comparable usage of water sources in which majority of the urban producers (71.8%) obtained water from pipe water whereas, farmers (45.8%) in the crop livestock system water in shashemane area used river water, while 24.8% from pipe water, 10.8% lake water, 10% spring water, and the rest 8.4% other sources. This reveals that there was better knowledge of the urban dairy cattle holding households on the importance of water to dairy cattle than the peri-urban dairy cattle holding households. There is need of awareness creation regarding water sources and frequencies of watering especially in the peri-urban areas so as to maximize milk production from the dairy cattle.

Livestock production experts and veterinarians during group discussion also expressed that animal health service provision is constrained by various restraining problems; absences of enough animal health clinics and inadequate trained animal health professionals were among others. In addition, the existing animal health clinics are not well equipped with the necessary materials, equipment and drugs to provide services at their full potential. Farmer's consciousness in maintaining animal health is negligible and this coupled with the above mentioned problems has reduced the efficiency of animal health service provision in both the urban and peri-urban areas. Moreover, the reason why higher incidences of mastitis reported in urban farms might be due to the presence of large and medium farms in urban areas and hence the exotic high yielder dairy cattle that are more susceptible to were also in those farms.

Wastes such as urine, wastewater, and feed leftover were removed either manually as was the case in small and medium farms or through concrete drainages in the case of large farms. Similarly Yousuf (2003); Moses *et al*. (2004) and Yitaye *et al*. (2009) reported that, in the urban areas manure collection, transport and disposal were generally chaotic. However, urban farmers were obliged to pile the cow dung outside of the farm which caused a nuisance to the area, including the risk of local pollution due to nutrient leaching. But in the peri-urban areas,

due to alternative uses of manure as organic fertilizer, waste disposal was not well thought-out as a serious problem. In addition to this, dung was made in to cakes and sold for fuel or used by the households as plastering material for their houses. Hence, sound waste management systems should be implemented by dairy farms in order to maximize the beneficial effects and also reduce its adverse effect on the environment especially in the urban areas. Manure from the urban areas is also supplied to a limited extent to the peri-urban areas, particularly to crop producing farms. Hence, urban-peri-urban linkage is evolved informally at the moment and this should be recognized and strengthened to benefit both urban and peri-urban dwellers in taking advantage of the chain. Therefore, contribution of manure produced as organic fertilizer was, thus, found economically important to both dairy farm owners and even to the surrounding rural farmers as 10% and 18.75% of the interviewed dairy farmers in urban and peri-urban, respectively, were using manure as source of their immediate income.

CONCLUSION AND RECOMMENDATIONS

The urban farmers depend on purchased feed sources but there is better usage of home grown feeds in the peri-urban areas. In the urban areas, pipe water was the most common source of water for dairy animals. But in the peri-urban areas, river played great as source of water for the dairy cattle. The peri-urban dairy keepers trekked their animals to get veterinary service to longer distance as compared to the urban dairy producers. Hence, establishing animal health clinics and equipping them with the necessary facilities, drugs and animal health professionals could be important to identify, control and monitor dairy cattle diseases and parasites in the study area particularly in the peri-urban areas. Forage development strategies and feed conservations should be encouraged in peri-urban whereas, establishing feed processing plants are recommended in urban areas. All these showed that, interventions need to correspond to the specific needs of the dairy farmers in urban and peri-urban areas.

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Original Article

Effects of Vitamin C, *Echium Amoenum* and Lavender Extract on Blood Metabolite and Meat Quality of Broiler Chickens Under Transport Stress

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ABSTRACT

A study was conducted with broiler chickens to determine the effect of some additives in drinking water on transport stress. Two hundred forty Ross 308 broilers aged 35 d were randomly assigned to 8 treatments with factorial arrangement (2×2×2) with 3 types of additives (vitamin C, echium amoenum and lavender extract) and tow levels (0 and 1200 ppm per liter of drinking water). Each treatment consisted of 4 replicates with 8 birds in each. On d 43, after collecting blood from the brides (2 birds from each replicate), all birds were transported (2 h under 8˚c temperature), then blood recollected. After slaughtering breast and thigh meat pH and water loss detected. Results showed that transport stress decreased blood glucose (mg/dl) and LDL (mg/dl), heterophile, H: L ratio but increased HDL, lymphocytes, eosinophile and heamatocrite of transported chickens ($P<0.05$). Additives didn't have significant effect on glucose, Cholesterol, triglyceride, HDL and LDL ($P>0.05$). Combination of 3 dietary supplements significantly decreased Heterophiles and H: L ratio of transported Birds ($P<0.05$). Echium amoenum and lavender extract were significantly lowered the breast meat pH. Broiler chickens which get lavender extract and Vitamin C have the highest L* and the lowest a* and lowest b* values belonged to vitamin C treatment birds ($P<0.05$). Drip losses of breast meat appeared to be significantly ($P<0.05$) lower in the combination of three dietary treatment received birds. ($P<0.05$). It is concluded that transport induced the reduction of blood glucose and LDL, heterophil, H: L ratio which are indexes of the stress in broiler chickens and combination of 3 supplements alleviate the adverse effects of transport stress.

Keywords: Transport stress, Herbal extract, Broiler, Blood Metabolism, Meat Quality.

INTRODUCTION

Domestic animals are invariably transported for a variety of reasons, including breeding, biomedical purposes, and slaughter, which exposes animals to potential stress and induces various psychological, physiological, and metabolic changes (Fazio and Ferlazzo, 2003, Fazio *et al.*, 2005). Transport stress reduces the animals' live weight gain (Kannan *et al.*, 2000, Fazio et al., 2005) and the quality of animal products (Pérez *et al.*, 2002), increases the animals' susceptibility to diseases (Hansson *et al.*, 2005) and impairs the animals' immune function (Stanger *et al.*, 2005). Transport stress can cause huge economic losses to the poultry industry because of stress-induced injuries, bird's mortality, and poorer quality of produced broiler meat (Voslářová *et al.*, 2007). Thus, the importance of reducing transport stress adverse effects on meat quality and improving broiler welfare is becoming widely recognized.

Undoubtedly, the appearance of meat is a critical factor influencing the desire of consumers to purchase meats and ultimately their satisfaction. Meat quality of domesticated animals can be affected by several ante-mortem stressors (Kannan *et al.*, 1997), one of which is pre-slaughter transportation. Transport alters both the metabolism and psychological state of animals, which may produce undesirable changes in meat quality (Owens and Sams, 2000). Concentrations of certain plasma metabolites such as cholesterol and glucose have been suggested to be sensitive parameters indicating the level of stress and muscle damage in poultry, also it stimulates glucagon release, which increases lipolysis and raises plasma concentrations of nonesterified fatty acids (Savenije *et al.*, 2002, Nijdam *et al.*, 2005, Huff *et al.*, 2010). The induction of physiological stress by transportation apparently activate the hypothalamo-adenohypophyseal-adrenocortical axis and it is consistent with the observation of post-transport increases in heterophil:lymphocyte ratios (Maxwell, 1993).

In poultry, the quality of meat products results from complex interactions between the genotype and the environment, more especially the stresses undergone before slaughter (Debut *et al.*, 2003). Pre-slaughter stressed animals have usually high temperatures, rapid glycolysis (pH drop), and early onset of rigor mortis in their muscles. Although the postmortem changes are rapid, some degree of ante-mortem muscle temperature rise, lactic acid buildup, and depletion of ATP also occurs. This combination of conditions results in an exaggeration of the muscle-to-meat transformation (rapid pH decline and an elevated carcass temperature resulting in protein denaturation) that normally occur. Muscles from pre-slaughter stressed birds usually become pale, soft, and moist or exudative (PSE) after a normal 18 to 24 h chilling period condition. This condition most often results lower possessing yields, increased cooking losses, and reduced juiciness (Froning and Uijttenboogaart, 1988). Ante-mortem stress, including heat-stress struggle before slaughter have shown to accelerate glycogen depletion, increase the rate of pH decline and possibly results in tough meat (Papinaho *et al.*, 1995). Again, Glycogen deficiency usually occurs when animal survive stress, such that associated with fatigue, exercise, fasting, excitement, fighting or electrical shock but are slaughtered before they have sufficient time to replenish their muscle glycogen stores. Muscle glycogen deficiency in these birds' results in limited glycolysis in the muscles after death and results in a high ultimate pH. As a consequence of a high ultimate pH, changes in muscle color that otherwise occur during the post-mortem transformation of muscle to meat, do not occur. The ultimate pH, color, and water holding capacity of broiler meat, and meat was paler in birds that underwent a commercial 2-h journey than in birds that were created for only 10 min and not transported (Kannan et al., 2000). These reports suggest that transport stress can influence the color and texture of broiler meat.

Increased antioxidative status in the living animal and a following increased oxidative stability of the raw product is considered beneficial for both the consumer and the processing industry. Feeding and conditions under which the animals are produced and slaughtered may influence the oxidative stability of the meat. Studies have mainly focused on the effects of

medicinal and aromatic plants on mortality; stress hormone levels, blood and muscle metabolism, meat quality and even immune function of domesticated animals although there is very little evidence clarifying how using medicinal plants before and during transportation affect metabolism and meat quality. Ascorbic acid may reduce the stress induced response, which follows the inevitable environmental stress imposed on the animals in connection with transport and slaughter procedure. Plants, such as tea (Tang *et al.*, 2000), rosemary and sage (Lopez-Bote *et al.*, 1998), containing high concentrations of antioxidants have also been demonstrated to reduce lipid oxidation in chicken muscle, and substantial antioxidative activity has been shown for Lavender in various in vitro model systems (Dorman *et al.*, 2000). The aim of the present work was to study some antioxidative defense mechanisms and their importance for blood metabolite; blood cell concentration and meat quality parameters such as color, pH and drip loss fluid.

MATERIALS AND METHODS

Chickens, Diet and Management

Broiler chickens Ross 308 were obtained from a commercial broiler chicken farm at 28 d of age and raised at our local facility under standard conditions with free access to water and feed. A total of 240 chickens were used for blood metabolites, cell concentration and meat color and pH measurements (see below). All chickens were weighted and assigned to dietary treatments based on equal average body weight and fed a basic diet as used for commercial production of broilers. Room temperature was 21°C during the first 35-45 d period. The relative humidity was maintained at 70% and a photoperiod of 20L: 4D was used. In order to accustom the birds to our farm condition, they were raised with commercial nutrients from 28-32. Broiler chickens were assigned to 8 dietary treatments, consisting 4 replicates of 8 birds each, according to a randomized complete block design with factorial arrangement (2×2×2). Experimental factors consist of 2 levels (0 and 1200 ppm per liter of drinking water) of 3 types of additives (vitamin C, *echium amoenum* and lavender ethanolic extract). The birds were kept under conventional conditions for temperature, ventilation, and lighting based on Ross catalogue recommendations (Ross, 2009) and were fed experimental diets from 33 to 45 d of age (table 1).

Table 1- Ingredients and nutrient composition of finisher diets

Ingredient (%)	Percent	Nutrient composition	
Corn	66.23	AME (MJME/Kg DM)	13.02
Soybean meal	27.69	Crude Protein %	18.00
Vegetable oil	1.00	Lysin (SID)[2] %	0.90
Fish meal	2.00	Meth (SID) %	0.40
DL-Methionin	0.13	Cys (SID) %	0.26
L-Lysin	0.10	Meth + Syc (SID) %	0.66
Di-Calcium Phosphate	0.90	Thr (SID) %	0.61
Oyster shell	1.05	Arg (SID) %	1.13
Salt	0.30	Ca %	0.86
NaHCO3	0.05	P %	0.52
Vitamin premix[1]	0.25	Na %	0.20
Mineral premix[2]	0.25	Cl %	0.23
		DCAB meq/Kg	204
		Linoleic Acid%	1.50
		Fiber %	4.33

[1]Each kg of vitamin premix provided: vitamin A 13 500 i.u., vitamin D3 2 000 i.u., vitamin E 30 mg, vitamin K3 2 mg, vitamin B1 1 mg, vitamin B2 6 mg, niacin 30 mg, pantothenic acid 12 mg, vitamin B6 3 mg, vitamin B12 10 µg, biotin 0.1 mg and choline chloride 500 mg.
[2]Each kg of mineral premix provided: Fe 50 mg, Cu 8 mg, Mn 80 mg, Zn 60 mg, I 0.5 mg, Co 0.2 mg, Se 0.15 mg, monensin sodium 100 mg and flavophospholipol 3 mg
[3]SID= Based on Standardized ileal digestibility.

Transport stress and biochemical parameters detection

At the age of 45d from 5 broilers from each treatment, blood samples were collected and blood metabolites like glucose, cholesterol, total protein, LDL-cholesterol and HDL-cholesterol were analyzed and white blood cell concentration like heterophile, lymphocyte and heterophil to lymphocytes ratio (H:L ratio) were calculated. After blood collection they crated, put in the baskets and transported for 2h at 25˚C, after coming back, blood samples recollected and blood metabolites and cell concentration again were analyzed. After that broilers were killed and hung by the legs for approximately 10 min to bleed out. Thereafter they followed standard processing conditions then burs and thymus glands also breast and thigh meat samples were collected. Bursa and thymus percentage were calculated based on body weight of broiler chickens.

Analytical Methods

Biochemical Examinations

The heparinized blood was centrifuged at 837 × g at 4°C for 10 min, and plasma samples were stored at −80°C in Eppendorf test tubes until the analyses were performed. Selected plasma biochemical indices (glucose, cholesterol, LDL, HDL, triglycerides and total protein) were measured by a Cobas Emira Biochemical Analyzer using commercial test kits (BioVendor Laboratorni medicina a.s., Modrice, Czech Republic).

Color Measurements

The (CIE, 1978) system color profile of L*, a*, and b* was measured by a reflectance colorimeter (Minolta Chroma Meter CR-300, Minolta Italia S.P.A., Milano, Italy) using illuminant source C. For breast (pectorals major) and thigh meat color evaluation, measurements were taken on the cranial, medial surface (bone side) in an area free of obvious color defects (bruises, discolorations, hemorrhages, full blood vessels, or any other condition that may have affected uniform color reading).

pH Measurement

The pH was determined using a modification of the iodoacetate method initially described by (Jeacocke, 1977). Approximately 2.5 g of breast and thigh meat was removed from the cranial end of each fillet, minced by hand, homogenized in 25 mL of a 5 mM iodoacetate solution with 150 mM potassium chloride for 30 s, and the pH of the homogenate was determined using a pH meter (pH meter HI98240 equipped with electrode FC230, Hanna Instrument S.p.A., Padova, Italy) calibrated at pH 4.0 and 7.0 at 45 minute and 24h after slathering.

Drip Loss Determination

The 2 fillets from each whole breast and thigh were separated and used for the determination of drip and cook loss. Drip loss was carried out on 1 intact fillet kept suspended in a sealed glass box for 48 h at 2-4°C and expressed as percentage of weight loss during storage.

Statistical Analysis

All measured criteria were analyzed by two-way ANOVA using GLM of (SAS, 2001) with Vitamin C, *echium amoenum* and lavender extracts as main effects. Comparisons of means of dietary treatments were done by Duncan's multiple range tests at the confidence interval of 95% ($P<0.05$).

RESULTS AND DISCUSSION

Blood Metabolites

Serum biochemistry is a labile biochemical system which can reflect the condition of the organism and the changes happening to it under influence of internal and external factors (table 2 and 3). Results of this study showed that both of the dietary treatments and transport stress did not have significant effects on blood biochemical parameters ($P<0.05$). The concentration of glucose, cholesterol and TG as major indicators of transport stress did not influence by dietary treatments although transport stress change them drastically (glucose, cholesterol and TG decreased) although in all of the treatments reduction amount of these parameters were equal (tables 2 and 3).

Table 2- Effects of dietary treatments on blood metabolites before transport stress

Treatments	Glucose	Cholesterol	TP	TG	HDL-Ch	LDL-Ch
Control	222.71	90.13	3.32	53.76	36.32	43.06
Vit. C	207.27	75.44	3.63	62.72	21.52	51.38
Lavender	220.72	92.54	3.61	50.29	24.16	58.33
E. A.	234.46	102.19	3.44	61.85	29.60	60.22
Vit. C + Lavender	229.38	103.51	3.35	65.32	25.28	65.17
Vit. C + E. A.	236.55	98.46	3.39	58.96	39.76	46.91
Lavender + E. A.	220.92	85.53	3.42	51.73	48.88	41.73
Vit. C + Lavender + E. A.	239.56	83.77	3.71	51.12	48.88	30.97
SEM	41.17	12.31	0.48	12.18	12.17	11.23
P-Value	0.60	0.50	0.56	0.88	0.31	0.91
ANOVA	*P-Value*					
Vitamin C	0.48	0.79	0.31	0.41	0.69	0.78
Lavender	0.31	0.98	0.24	0.78	0.39	0.86
E. A.	0.51	0.81	0.32	0.98	0.06	0.25

Means within a column with no common superscript are significantly different ($P < 0.05$)
TP= total protein, TG= triglyceride, Ch= cholesterol, Vit= vitamin, E.A= echium amoenum, LDL= low density lipoprotein, HDL= high density lipoproteins.

The burse and thymous percentage were not affected by dietary treatments ($P> 0.05$). After slaughter, the substrates glycogen, glucose, and glucose-6-phosphate are converted to lactate. As is shown in the study by (Ondrašovičová *et al.*, 2008), rough handling and long journeys have the greatest adverse effects on poultry welfare. They found that the time in transit and the distance between the farm and the slaughterhouse increased the corticosterone level in plasma and reduced the glucose level in blood.

Similarly, (Pijarska *et al.*, 2006) detected a lower glucose concentration in birds after transportation lasting 18 h. Also triglycerides, total protein and glucose levels of broilers decreased with travel distance so that their levels were decreased after 130 km of transport when compared with broilers before transport at fall and winter temperatures (Vosmerova *et al.*, 2010). At the other hand no significant differences in plasma glucose or lactate concentrations in broilers transported for 1.5 h were found. (Savenije et al., 2002, Delezie *et al.*, 2007).

Dietary essential oils like borneol, cineole, citral, geraniol, menthone, menthol, fenchone and β–ionone suppresses the hepatic HMG–CoA reductase activity (Yu *et al.*, 1994). The hypo-cholesterolemic effect of lemongrass oil is due to the inhibition of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG–CoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis (Cooke *et al.*, 1998, Crowell, 1999). Significant reduction in the serum cholesterol level of broiler fed with cinnamon powder at 250 and 500 ppm (97.43 and 94.87 mg/dl vs 116 mg/dl) were reported (Gopi *et al.*, 2013).

Table 3- Effects of dietary treatments on blood metabolites after transport stress (mg/dl)

Treatments	Glucose	Cholesterol	TP	TG	HDL-Ch	LDL-Ch
Control	91.83	104.39	3.11	55.20	40.96	52.39
Vit. C	113.15	86.40	3.61	46.84	44.16	32.88
Lavender	149.10	98.25	3.80	45.95	37.52	51.53
E. A.	146.24	105.70	3.28	48.84	63.04	32.98
Vit. C + Lavender	120.12	104.61	3.84	51.73	71.60	31.58
Vit. C + E. A.	120.92	98.25	3.38	44.51	62.88	26.46
Lavender + E. A.	129.98	92.54	3.70	49.71	56.80	25.19
Vit. C + Lavender + E. A.	157.37	111.18	3.81	57.80	53.76	45.80
SEM	22.96	10.61	0.34	7.28	10.12	10.79
P-Value	0.92	0.95	0.13	0.93	0.25	0.38
ANOVA	*P-Value*					
Vitamin C	0.72	0.98	0.47	0.96	0.25	0.41
Lavender	0.46	0.69	0.76	0.32	0.15	0.23
E. A.	0.14	0.64	0.02	0.95	0.76	0.74

Means within a column with no common superscript are significantly different ($P < 0.05$)
TP= total protein, TG= triglyceride, Ch= cholesterol, Vit= vitamin, E.A.= echium amoenum, LDL= low density lipoprotein, HDL= high density lipoproteins.

The broilers showed a dose dependent reduction in serum cholesterol levels. Poultry are renal synthesizers of vitamin C, but its quantity becomes insufficient under praxis conditions as a result of increased rate of usage in combating the free radicals thus generated (Gopi et al., 2013). In the other study researcher reported that separately or as a combination, supplemental vitamin C and E decreased plasma concentrations of cholesterol and glucose of laying hens reared under condition of high ambient temperature and humidity(Ajakaiye *et al.*, 2010). Furthermore, the supplementation of vitamin C and E have been reported to increase serum concentration of total protein, but decrease corticosterone, glucose, and cholesterol concentrations in Japanese quails exposed to 33 °C (Sahin *et al.*, 2003). Several authors have documented (Altan *et al.*, 2003, Imik *et al.*, 2009) that free radical generation affects blood serum metabolites of total protein, cholesterol and glucose which is manifested in bird's adaptation response through decreased production performance. Vitamin C has been demonstrated to be a powerful antioxidant that acts through a two way mechanism, those are, through its conversion to L-dehydroascorbic acid and formation of an ascorbate radical, a particularly inert radical, this reaction is reversible and the interconversion of these molecules forms a redox system which is the basic physiology of their actions, because both show vitamin C activity (Yildiz *et al.*, 2009). In the present study, vitamins C and lavender or borage supplementation did not improve the stability of serum metabolites of broiler chickens under transport stress. Increases in concentrations of glucose during stress may be attributed to induced glucocorticoid secretion which increases glucogenesis. Glucocorticoids are well known not only as hormones essential for maintaining life and normal growth, but also as stress hormones. Dietary vitamin C, lavender and borage extracts may reverse these changes, probably by reducing the secretion or synthesis of glucocorticoids (McDowell, 2008). These results are agreement with our study, in which we found no significant effect of transport stress on the glucose level in broilers. The results obtained indicate that pre-transport treatment (dietary supplementation) may be less useful for broilers than bettering handling procedures (catching, crating in good condition and loading) in order to reducing transport stress effect on blood biochemical parameters.

White Blood Cell Counts

White blood cell differential counts are summarized in Table 4. Before transport stress, vitamin C and combination of extracts with citamin C significantly decreased heterophil and increased Lymphocyte percentage as compared with control group but after transport stress, addition of vitamin and plant extracts alone or in combination with the other compounds significantly decreased heterophil percentage and H: L ratio and increased Lymphocyte percentage as compared with control group ($P< 0.05$). In general, animals respond to transport stress by increasing the number of total WBC and specific types of WBC (heterophils, eosinophils, and mononuclear cells) in circulation (Kent and Ewbank, 1986). Heterophil counts, which are sensitive indicators of changes in plasma corticosteroid levels and extent of stress (Post *et al.*, 2003) were highest in control group birds after transport stress and among the dietary treatments birds those received all of the additives had lowest heterophil percentage and this led to a significant decrease in H/L ratio after stress. It is suggested that H/L ratios of 0.2, 0.5, and 0.8 characterize low, optimum, and high levels of stress, respectively. In this study, H/L ratios were significantly varies among the preslaughter treatments, most likely due to long and sever duration of the transport stress (Gross and Siegel, 1982) and significant increase in H/L ratio in broilers after extended (actual transport time was 3 h) transportation were reported (Mitchell *et al.*, 1992). In conclusion, in this study substitution of medicinal plant extracts beside vitamin C because of their antioxidant effect could reduce the detrimental effect of transport stress on white blood cell count and decrease the heterophil percentage in blood of broiler chickens.

Table 4- Effects of dietary treatments on blood cell population before and after transport stress

	Before stress			After stress		
Treatments	H%	L%	H/L	H%	L%	H/L
Control	28.25^{b}	60.25^{c}	0.469^{ab}	35.75^{a}	52.75^{f}	0.683^{a}
Vit. C	26.25^{bc}	66.00^{b}	0.398^{ab}	19.50^{b}	72.25^{cbd}	0.271^{cb}
Lavender	22.25^{cd}	72.75^{a}	0.306^{cd}	14.00^{de}	75.00^{b}	0.187^{de}
E. A.	33.5^{a}	60.00^{c}	0.563^{a}	17.50^{bc}	71.25^{ced}	0.246^{bcd}
Vit. C + Lavender	33.5^{a}	61.25^{c}	0.549^{a}	20.75^{b}	68.00^{e}	0.307^{b}
Vit. C + E. A.	21.75^{d}	71.50^{a}	0.311^{cd}	15.50^{cd}	74.75^{cb}	0.208^{cde}
Lavender + E. A.	33.5^{a}	60.75^{c}	0.553^{a}	19.25^{b}	69.50^{de}	0.277^{bc}
Vit. C + Lavender + E. A.	20.00^{d}	71.50^{a}	0.281^{d}	11.00^{e}	78.75^{a}	0.141^{e}
SEM	1.39	1.54	0.03	1.11	1.18	0.024
P-Value	0.009	0.009	0.001	0.001	0.001	0.001
	P-Value			*P-Value*		
Vitamin C	0.004	0.001	0.001	0.001	0.001	0.001
Lavender	0.72	0.07	0.64	0.001	0.001	0.001
E. A.	0.93	0.43	0.82	0.001	0.001	0.001

Means within a column with no common superscript are significantly different ($P < 0.05$)
Vit.= vitamin, E.A.= echium amoenum, H= heterophil, L= lymphocyte, H/L= H to L ratio

Meat Quality Parameters

Table 5 shows the effects of treatment on initial and final pH, drip loss and CIE Lab color coordinates on breast and thigh samples. Addition of plant extracts and vitamin C one week before transport significantly influenced meat quality characteristics such as initial and final pH and color of breast and thigh meats. Treatment also influenced the drip loss of breast meat, but it did not influence the drip loss level of thigh meat ($P < 0.05$). Mean initial pH of breast meat of the birds those received echium amoenum extract was significantly lower ($P < 0.05$) than that of the other dietary treatment birds and the final breast meat pH of lavender received

birds was lower than that of others. In the case of thigh meat there was a little inconsistency in initial and final pH and it was difficult to describe the results. The color indexes (L*, a* and B*) of breast meat were differ between treatments so that birds which get lavender extract and Vitamin C have the highest L* and the lowest a* and lowest b* values belonged to vitamin C treatment birds ($P<0.05$). No effects of the dietary treatments were found for L* of thigh meat ($P>0.05$). In contrast, a significant differences between treatments were observed for a* and b*, with lower values in the birds those received lavender extract and also vitamin C and echium amoenum ($P<0.05$). Drip losses of breast meat appeared to be significantly ($P<0.05$) lower in the combination of three dietary treatment received birds. The antioxidative properties of various extracts of plant oils like oregano, thyme, marjoram, spearmint, lavender and basil which were assessed by addition them to lard kept at 75 °C and found out that extract containing Oregano was the most effective followed by thyme, dittany, marjoram and lavender (Economou *et al.*, 1991, Raza *et al.*, 2009). However, ΔpH tended to be high in plant extract fed birds than control birds, indicating that plant extracts and vitamin C because of their antioxidant activity may slow the pH decline and help maintain meat quality.

This variation in meat quality may be related to the chemical changes in muscle myoglobin pigment, which is predominantly converted into purple reduced myoglobin and brown metmyoglobin during the first days postslaughter (Millar *et al.*, 1994) in chickens, (Sante *et al.*, 1993) in turkeys. There was an indication of a positive relationship between plasma corticostrone (CORT) concentrations and color of thigh meat and an increase in CORT concentration is associated with a higher hue value, indicating that the meat becomes lighter and less red in color (Kannan et al., 2000). This result suggests that very high stress levels in broilers may cause production of paler thigh meat. It is likely that different preslaughter stressors can affect the metabolism of different fiber types (red, white or intermediate) in animals (Fernandez *et al.*, 1994).

CONCLUSION

According to this results of this study it is concluded that using lavender and echium amoenum extract and vitamin C as natural and synthetic anitioxidants can reduced adverse effects of transport stress by lowering heterophil percentage and H; L ratio, those are the indexes of stress in broiler chickens also they prevented the effect of long period transport stress on breast and thigh meat quality by elevating the l* value and lowering the a* and b* values of these meats. At the other hand transport stress had unfavorable affect on blood metabolites and using synthetic and organic antioxidants compensate these effects on blood metabolites of transported broiler chickens. It is suggested that antioxidant can alleviate the adverse effect of transportation on blood metabolite and meat quality and in some cases we can use of lavender and echium amoenum extract beside vitamine C in order to reducing transport stress bad effects on meat quality.

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Table 5- effects of dietary treatments on broiler chicken meat quality of after transport stress

	Treatment[1]												
Meat	A	B	C	D	E	F	G	H	SEM	*P-Value*	*P-Value*[2]		
Breast Color[3]											V	L	EA
L*	46.88^{b}	48.38^{ab}	56.68^{ab}	48.38^{ab}	58.60^{a}	56.67^{ab}	49.34^{ab}	50.97^{ab}	3.55	0.001	0.59	0.82	0.81
a*	12.29^{a}	9.60^{ab}	8.68^{b}	10.44^{ab}	8.85^{b}	10.36^{ab}	10.82^{ab}	10.19^{ab}	1.02	0.001	0.073	0.64	0.28
b*	6.08^{ab}	4.18^{b}	9.23^{a}	8.33^{a}	9.24^{a}	9.11^{a}	7.65^{ab}	9.16	1.26	0.001	0.11	0.12	0.72
pH 45min	6.78^{a}	6.50^{abc}	6.57^{abc}	6.20^{c}	6.39^{abc}	6.62^{ab}	6.63^{ab}	6.36^{bc}	0.12	0.028	0.37	0.016	0.38
pH 24 h	6.02^{ab}	6.24^{a}	5.92^{b}	6.13^{ab}	6.07^{ab}	5.99^{ab}	5.97^{ab}	6.36^{bc}	0.08	0.04	0.54	0.14	0.59
ΔpH	0.76^{a}	0.41^{bc}	0.27^{c}	0.44^{abc}	0.35^{bc}	0.63^{ab}	0.59^{abc}	0.39^{bc}	0.10	0.010	0.35	0.03	0.38
D.L. %	2.18^{c}	2.45^{c}	2.65^{bc}	2.70^{bc}	3.43^{bc}	3.742^{ab}	2.988^{bc}	4.73^{a}	0.39	0.032	0.45	0.44	0.11
Thigh Color													
L*	44.54	44.81	54.64	52.14	46.14	52.34	47.54	51.75	3.72	0.228	0.71	0.20	0.56
a*	17.07^{a}	15.47^{abc}	13.49^{bc}	16.77^{ab}	16.59^{ab}	13.06^{c}	13.45^{bc}	14.89^{abc}	1.05	0.003	0.42	0.14	0.19
b*	4.08^{bc}	8.98^{a}	3.28^{c}	9.26^{a}	7.81^{ab}	6.17^{abc}	5.94^{abc}	6.12^{abc}	1.29	0.036	0.52	0.82	0.15
pH 45min	6.632^{b}	6.91^{a}	6.58^{b}	6.595^{b}	6.51^{b}	6.52^{b}	6.58^{b}	6.52^{b}	0.08	0.039	0.77	0.06	0.08
pH 24 h	6.28^{ab}	6.60^{a}	6.29^{ab}	6.56^{ab}	6.40^{ab}	6.38^{ab}	6.24^{b}	6.24^{b}	0.10	0.018	0.36	0.03	0.61
ΔpH	0.35	0.30	0.29	0.04	0.11	0.13	0.34	0.27	0.11	0.532	0.84	0.36	0.63
D.L. %	1.61	1.54	1.64	1.97	1.69	2.33	2.29	2.57	0.40	0.768	0.22	0.04	0.41

Means within a row with no common superscript are significantly different ($P < 0.05$)

1- A= Control, B= Vitamin C , C= Lavender, D= echium amoenum, E= Vitamin C + Lavender, F= Vitamin C + echium amoenum, G= Lavender + echium amoenum, H= Vitamin C + Lavender + echium amoenum,

2- V= Vitamin C, L= Lavender, EA= echium amoenum, VLEA= Vitamin C + Lavender + echium amoenum

3- L* = Lightness; a* = Redness; b* = Yellowness, D.L.= drip loss

4- ΔpH= pH at 45 Min Postmortem - pH at 24 h Postmortem.

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Review Article

The Emerging Nutricitical Benefits of the African Wonder Nut (Garcinia Kola Heckel): A Review

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ABSTRACT

Garcinia kola also called bitter kola and African wonder nut has been shown to contain vitamins, minerals, proteins and carbohydrates in varying quantities and phytochemicals such as alkaloids, flavonoids, tannins, cyanogeinic glycosides, saponins among many others that give its characteristics nutritional and pharmaceutical properties. The use of *Garcinia kola* as a nutritional plant will depend on the nutritive value of the seed and other parts, its overall effect on animals performance and human health as well as market forces affects on its demand and supply. This Paper reviews published data on the nutritive and pharmaceutical values of *Garcinia kola* as it affects the health and performance of human beings and animals.

Keywords: Emerging Nutricitical, African Wonder, Garcinia Kola

INTRODUCTION

New initiatives in the livestock and pharmaceutical industries are seeking to promote the use of alternative materials that combine the effects of nutritional and medicinal properties, simultaneously. This is expected among others benefits to reduce the high cost of production in the livestock industry as a result of the reduction in dual costs of feed and drugs. One important component of this approach is to research into indigenous fruit trees or plants that possesses both nutritional and medicinal properties. While a lot of research attention has been paid to the pharmaceutical benefits of these plants, little attention has been paid to their potential as feed ingredient in the livestock industry, although research has been carried out to

assess the nutrient compositions of some of them. Earlier studies at our station have shown that leaves from *Alchornia cordifolia* (Udedibie and Opara, 1998) and *Azadirchta indica* (Esonu *et al*., 2005; Obikaonu, 2009) could be of value in poultry diets.

Garcinia kola (bitter kola) also known as African wonder nut belongs to the family *guttiferae* and grows in coastal rainforests in the South-Western and South-Eastern parts of Nigeria. Traditionally, the nuts of *Garcinia kola* are chewed as masticatory substance to stimulate the flow of saliva (Leakey, 2001). The kernels of the nuts are widely traded and eaten as a stimulant (Omode *et al*., 1995; Leakey, 2001). *Garcinia kola* is also highly valued because of its medicinal benefits (Hertog *et al*., 1993). The nuts are chewed for aphrodisiac effect or used to cure cough, dysentery or chest cold in herbal medicine (Irvine, 1961).

This review seeks to aggregate current information on the characteristics of *Garcinia kola* both as a nutritional and medicinal plant of emerging importance.

Taxonomy of *Garcinia kola*

Bitter kola known as *Garcinia kola heckel* is scientifically classified as follows:

Kingdom: *Plantae*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Theales*
Family: *Clusiaceae or guttiferae*
Genus: *Garcinia*
Species: *G. kola*
Binomial name: *Garcinial kola, Heckel.*

About 400 species of *G. kola* are found in tropical regions especially in Asia and in South Africa (Mabberly, 1987). Some of these species are: *Garcinia cambogia, gaudichaudii, hanburyi, huillensis, indica, kola, latissima, mangostana, morella, oliveri* and *polyantha.* Other species of *Garcinia* found in Nigeria as well as generally across the humid low land plains of West Africa extending from Sierra Leone to Zaire according to Vivian and Faure (1996) and Angola (Keay,1989) include; *Garcinia livingstonei, gnetoides, standtii, smeath emanii, ovalivolia, brevipediellata* and *manni*

Origin and distribution of *Garcinia kola*

Bitter kola has also been recognized as an indigenous medicinal plant found in rain forest of central and western Africa, especially Benin, Cameroon, Democratic Republic of Congo, *Cote d'Ivoire*, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. Its natural habitat is thus the subtropical or tropical moist lowland forests. It is usually found in the coastal areas and low land plains up to 300 m above sea level with an average of 2500 mm of rainfall per annum. The major places where the plant would be found growing in the wild are forest reserves and free areas of the rain-forest or it is planted or conserved in on-farm oil-palm, cocoyam plantations. These growing regions have low altitude with annual temperature ranging from 32.15^{o}C to 21.4^{o}C and a relative humidity of 76.34% (Ntamag, 1997). In the coastal rainforests of south-western and south-eastern parts of Nigeria the nut is chewed and readily served to visitors as a sign of goodwill. Bitter kola is enjoyed by the three major ethnic groups in Nigeria (i.e. the Yoruba's, the Igbo's and the Hausa's), from whom it derived the local names *agbilu* in Igbo and *Orogbo* in Yoruba languages.

The local market for bitter kola extends beyond the southern production areas to the northern parts of the country. In Nigeria, its trade is as important as that of kola nut (*Cola nitida and C. acuminata*) in major towns and cities in southern parts of the country where the tree is endemic.

Botanic and Agronomic Characteristics

According to an earlier description by Heckel and Schlagdenhauffen (1884), bitter kola is a tree of variable aspect, well branched, ever-green and grows to a height of about 12m. Towards the base of the branches are large opposite leaves (12" long by 7" broad), with short petioles, while at the extremity of the branches, the leaves are much smaller (5" by 2"). The leaves are oval, slightly dilated at the base, full green on the upper surface and greenish underneath. Ladipo (1995) reported that the tree produces reddish yellowish or orange colored fruit, with each fruit containing two to four yellow seeds and a sour tasting pulp. The seeds when consumed have bitter astringent taste. The fruit is classified a berry, the size of an apple with a rugos epiderm covered entirely with rough hairs.

As a tropical fruit tree species, it is characterized by slow rate of growth (Ladipo, 1995). Cultivation of the plant is limited because of poor germination and the length of time it takes (about 10 - 15 years) to reach reproductive phase. In Nigeria, the demand for bitter kola is high but the production is limited due to problem of seed dormancy; untreated seeds are difficult to germinate. Farmers believe that germination of bitter kola takes about six to twelve months and that only a few seeds germinate. There is also the problem of setting up nurseries. However, Anegheh *et al.* (2006) developed a pre-nursery treatment to break dormancy and enhance germination. This work revealed that seed cutting (nicking) was very effective in enhancing germination of *Garcinia kola*. The seed is first raised in the nursery and then transplanted to the field.

Fruiting commences in July and ends in October, while harvest continues as ripe fruits fall and are collected for the extraction of seeds. Ladipo (1995) reported that a mature fruit tree of 10 to 15 years produces 85 to 1717 fruits, with 208 to 6,112 seeds. Taking the mean of these values at 834 fruits and 2,627 nuts per tree he projected a fruit production of 26 tonnes per ha per annum with 278 trees per hectare at 6 x 6m spacing.

When ripe, the green pericarp of the fruit turns reddish yellow color and the fruit falls. The fruits are gathered, broken and stored in an open cool place to allow for fermentation of the pericarp and pulpy mesocarp. Thereafter, they are threshed to release the seeds which are washed to remove the sticky mucilaginous material that sheaths them. The seeds or nuts that are not sold fresh are air dried and stored in baskets lined with jute bags. According to Ofor *et al.* (2010) storage of bitter kola in polyethene bags is favored in terms of shelf life and palatability.

Cultivation of *Garcinia kola*

According to *Ingenieurs Sans Engineers Frontieres without Boarders Cameroon* (2009). *Garcinia kola* is cultivated either by seeds or by cutting. Seed cultivation involves preparing a suitable seed bed measuring 3 x 4m ($12m^2$) on a flat ground inside a shade house to protect the little plants from direct radiation of the sun and strong rains. The shade is usually built from local materials like bamboo, stakes cut in forest or palm tree branches. Seeds that germinated easily are usually those from matured ripe fruits that fell to the ground before the seeds are removed.

To achieve pre-nursery germination, two or three banana or plantain tree trunks are cut and large holes made on the level of their bases to allow for destruction of the central bud (meristem) of the cut plant. Seeds are thereafter inserted into the trunks of the banana tree and the two ends of each trunk attached firmly. Trunks are then arranged hermetically closed under a hanger. After three months the binding wires are detached to recover the seeds which had already germinated.

The germinated seeds are carefully sown inside polyethylene bags with $^2/_3$ of it filled with a mixture of black soil and sand. The pots of young seedlings are then laid out in the seed bed, and maintained by watering every two days, weeding, application of 3g of N.P.K 20:10:10

fertilizer every 3 months and fighting off fungal and insect attacks with the appropriate fungicides and insecticides. After 12 months, the seedlings are planted in the field, usually at the beginning of the rainy season with a standard spacing of 10 x 10m. In the field, further agronomic practices are observed, and production of fruit will begin approximately after 7 years.

Cultivation by cutting is usually done using bitter kola cuttings obtained from very tender branches and stems with young healthy leaves and vertical branches looking upwards. Preferably, cuttings are best cut very early in the morning and just after rain to avoid drying. This is transferred into a wet plastic bag, which is tightly closed and kept under shade from where they are transferred directly to the propagator. Cutting are usually about 12cm. The young seedlings that germinate are carefully inserted into polyethylene bags filled with mixture of black soil and sand up to $^{2}/_{3}$ level. After insertion of the young seedlings, the polyethylene bags are filled with soil to the brim and sprinkled lightly with water. The pots of seedling are thereafter transferred to the seed bed and finally to the field as in propagation by seeds. *Garcinia kola* usually produces fruits between the months of July and October.

Traditional uses of *Garcinia kola*

Garcinia kola is cultivated throughout West Africa for its edible fruit and seeds which are used as rejuvenating agent for masticatory purposes and as a general antidote (Ibiblio, 1983). Among the Igbos of Nigeria it is presented to visitors as a sign of peace and welcome. It is also used to entertain guests during ceremonies and festivities. Again, it is popularly used among other Nigerian groups for nervous alertness and induction of insomnia when chewed.

Traditionally, the nuts of *Garcinia kola* have used as sialegogue to stimulate the flow of saliva (Leakey, 2001). The kernels of the nuts are widely traded and eaten as a stimulant (Omode *et al.,* 1995; Atawodi *et al.,* 1995; Leakey, 2001). It is believed to clean the digestive system, without side effects such as abdominal problems, even when a lot of it is eaten (Onochie and Stanfield, 1960). In traditional medicine, the dried nut is ground and mixed with honey to make a traditional cough mixture. The ground nut may also be mixed with water and given to new born babies to relieve stomach cramps.

Experimentations using *Garcinia kola* kernels as hop substitutes in several indigenous alcoholic drinks as well as flavour enhancer in the beverage industry also exist (FDA, 1999). Ofor *et al.* (2004) identified several ethno-botanical uses to which the indigenes of Imo state in South–eastern Nigeria put the *Garcinia kola* seeds. These include as an antidote to snake bites, poison and overdose, for cough, vomiting and as a snake repellent. The seeds which serve as a bitter stimulant also serve as a snake repellent when they are placed round the compound (Nair, 1990). The seed is used in the treatment of diarrhoea (Braid, 1991), bronchitis and throat infections (Orie and Ekon, 1993; Adesina *et al.,* 1995), liver disorders (Iwu *et al.,* 1990) and enjoys a folk reputation in Africa as a poison antidote (Kabangu *et al.,* 1987). According to Farombi *et al.* (2005), the seeds of *Garcinia kola* have pharmacological uses in treating coughs, throat infections, bronchitis, hepatitis and liver disorders.

The Plant

Every part of the *Garcinia kola* plant has been useful in traditional practice ranging from the root of the plant to its seed. The root of the plant serves as a bitter chewing stick in West Africa, while the stem serves as a chewing stick for many people in southern Nigeria (Olabanji *et al.,* 1996; Uko *et al.,* 2001; Okwu and Ekeke, 2003). The products of three *Garcinia kola* species are widely used in Ghana and 70% of its use is as chewing sticks. These are brought into urban markets as an alternative to tooth paste and brush (Adu-Tutu *et al.,* 1979). The raw stem bark serves as purgative, the powdered bark is applied to malignant tumours, the sap is used for curing parasitic skin diseases and the latex or gum is used against

gonorrhea infection and applied externally on fresh wounds to prevent bacterial contamination.

Other by-products of *Garcinia kola* plants are also useful to mankind. The wood makes excellent fuel source. Its dense rounded crown makes it an ideal tree for shade around homesteads. The branches are used as chewing stick because of its taste and anti-bacterial activities of its extract (Taiwo *et al.,* 1999).

Nutrient composition of *Garcinia kola* nuts

Garcinia kola contains nutrients such as proteins, carbohydrates, fiber, minerals, fat and oils. Ibekwe *et al.* (2007) reported that *Garcinia kola* seed has poor nutrient composition but highly valued in trado-medicine due to its useful active phytochemical composition.

Contrary to the nutrient compositions of *Garcinia kola* reported by Ibekwe *et al* (2007) in table 1, Esiegwu and Udedibie (2009) reported nutrient compositions of *Garcinia kola* as shown in table 2. Odebunmi *et al.* (2009) reported the moisture content of the seeds to be 60.48 $\pm$ 0.06%, dry matter of 39.52 ± 0.06%, crude fat of 4.51 ± 0.56%, crude protein of 2.48 ± 0.10%, ash content of 0.79 ± 0.005%, crude fiber of 5.23 ± 0.16% and total carbohydrates (+ fiber) of 35.64%. These values are different from what had previously been reported for bitter kola. Eleyinmi *et al.* (2006) reported a protein content of 3.95%, lipid of 4.33%, ash of 1.14% and a crude fiber content of 11.4% in the seed.

Table 1: Nutrient composition of Garcinia kola (% of dry matter)

Components	Amount%
Moisture	14.60
Crude protein	0.58
Crude fiber	0.10
Ether extracts	3.00
Ash	5.00
Nitrogen-free extract	91.32

Adapted from Ibekwe *et al*. (2007)

Table 2: Nutrient composition of *Garcinia kola* (% of dry matter)

Composition	Amount%
Dry matter	7.30
Crude protein	2.64
Crude fiber	20.51
Ether extracts	9.47
Ash	1.07
Nitrogen free extracts	57.54

Adapted from Esiegwu and Udedibie (2009).

Chemical composition of *Garcinia kola*

Chemical analysis of *Garcinia kola* seed in Nigeria as reported by Okwu (2005) showed that it contains a wide range of vitamins and minerals as shown in tables 3 and 4. According to Odebunmi *et al.* (2009), *Garcinia kola* has 722.10 mg/100g of potassium (K), 67.07±0.12 mg/kg DM of calcium (Ca), 114.83±3.47 mg/kg DM of magnesium (Mg), 6.10±0.43 mg/Kg DM of iron (Fe), 2.30±0.08 mg/kg DM of zinc (Zn), and 188.57±0.37 mg/kg DM of phosphorus (P).

Asaolu (2003) also reported that the fresh seeds of bitter kola (wet weight) contains high moisture content of 75.50% and dry weight of 24.50, while the ash content was found to be 5.90%, crude fat was 14.50%, carbohydrate was 10.85%, crude fat was 14.50% and crude

protein was found to be very low (4.25%). Dosunmi and Johnson (1995) in comparing the nutritive value of the fresh fruit from Nigeria showed that crude protein was higher in the mesocarp (7.8%) than in the pericarp (3.9%), while the pericap was richer in crude fiber (13.9% - 16.5%). The mesocarp was also richer in crude lipid (6.9% - 8.7%). Unsaturated fatty acids (linoleic acid, 40.5%, oleic acid, 30.8%) are the main components of the lipids (4.5%) found in the seeds of this species (Essien *et al.*, 1995; Omode *et al.*, 1995).

Table 3: Vitamin composition of *Garcinia kola* seeds (dry weight basis)

Vitamins	Amount (mg/100g)
Thiamin (vit. B1)	0.5 ± 40.30
Riboflavin (vit. B2)	0.22 ± 0.01
Niacin (nicotinic acid)	1.60 ± 0.01
Ascorbic acid (Vit. C)	23.10 ± 0.02

Adapted from Okwu (2005).

Table 4: Mineral composition of *Garcinia kola* seeds (dry weight basis)

Mineral	Amount (mg/100g)
Macro elements	
Magnesium	0.42 ± 0.30
Calcium	0.80 ± 0.40
Potassium	2.50 ± 0.10
Phosphorus	0.33 ± 0.10
Sodium	0.72 ± 0.10
Micro elements	
Iron	17.75 ± 0.30
Zinc	2.30 ± 0.01
Copper	0.78 ± 0.20
Manganese	2.01 ± 0.50
Chromium	0.00
Cobalt	0.55 ± 0.20
Cadmium	0.29 ± 0.10

Adapted from Okwu (2005).

Phytochemical Constituents of *Garcinia kola*

The role of phytochemicals in enhancing body cell immunity against diseases in the body cannot be overemphasized. The active constituents contributing to the protective effect of *Garcinia kola* on animals is attributed to the presence of phytochemicals, vitamins and minerals (Okwu and Ekeke, 2003). Phytochemicals exhibit a wide range of biological activities as a result of the anti-oxidant properties of some of these chemicals. Several types of polyphenols (phenolic acid, hydrolysable tannins and flavonoids) show anti-carcinogenic and mutagenic effects (Uruquiaga and Leighton, 2000). Okwu (2005) and Esiegwu and Udedibie (2009) reported the phytochemical values as shown in table 5 and 6.

Table 5: Phytochemical constituents of *Garcinia kola* seeds (Dry weight basis).

Constituents	Amount (mg/100g)
Phenols	0.11±0.20
Alkaloids	0.36 ± 0.10
Tannins	0.26 ± 0.20
Flavonoids	1.98 ± 0.20

Adapted from Okwu (2005).

Table 6: Phytochemical constituents of *Garcinia kola* seeds (dryweight basis)

Constituents	Amount (mg/100g)
Cyanogenic glycosides	0.54
Tannins	0.34
Saponins	10.06
Alkaloids	4.93

Adapted from Esiegwu and Udedibie (2009).

Garcinia kola stem has been shown to contain a complex mixture of phenolic compounds such as biflavonoids, xanthones and benzophenone (Iwu and Igboko, 1982) which have anti-microbial activity as kolanone (Hussain *et al.,* 1982), kola flavonone and garcinia flavonone (Iwu, 1993). Phytochemical studies have shown that the seeds constituents include biflavonoids, xanthones and benzophenones. Thus, the seeds of *Garcinia kola* are known to have a general antidote effect in traditional medicine in Africa. These possibly explain its reported aphrodisiac properties and in the treatment of catarrh and abdominal colicky pain. In addition, their use is believed to improve singing voice and relieve cough (Irvin, 1961).

The feed value of *Garcinia kola* for farm animals

Although *Garcinia kola* is eaten by many Nigerians and in most parts of Africa as an aphrodisiac, for the medicinal value or for pleasure, not much work has been done to determine its feed value for animals in terms of feed intake, growth rate and feed conversion ratio. However, Uko *et al.* (2001) reported that water extract from *Garcinia kola* administered to growing winstar rats in three doses of 0, 10, 20 mg/100g body weight of rats daily to respective group of 15 rats for a period of 70 days showed depressive effect on appetite and water intake with resultant poor feed utilization efficiency and weight gain of rats in a dose-dependent manner.

Contrary to the report of Uko *et al.* (2001), Esiegwu and Udedibie (2009) reported that *Garcinia kola* seed meal in broiler diets at 0, 2.5, 5.0 and 7.5% levels fed to groups of 30 broiler chicks for 8 weeks recorded no significant differences in feed intake among the groups but the group on 2.5% *Garcinia kola* diet had significantly heavier body weight and superior feed conversion ratio than the other groups. *Garcinia kola* has being reported to improve digestibility when chewed in small pieces before any meal (Kafaru, 1998). Improved digestibility could enhance intake and consequent gain in weight. In a very recent study Esiegwu *et al.,* (2012) reported that there was no treatment effect on body weight, feed intake, feed conversion ratio and egg quality indices of laying hens fed graded levels of *Garcinia kola*. However, histological alterations of the kidney, liver and gizzard were observed.

Effect of *Garcinia kola* on haematological and serum biochemical indices.

Uko *et al.* (2001) reported that water extract from *Garcinia kola* administered to rats did not cause any significant differences between blood samples from the control and experimental rats for HB, PVC, RBC and erythrocyte indices. However, there was a general inverse relationship between the erythrocyte values (HB, PVC and RBC) and increased doses of the plant extract. There was also a significant increase in total leucocytes count of blood samples from the experimental rats, which depended on the doses of the extract. The work also showed that bitter kola extract decreased total plasma proteins, albumin concentrations but slightly increased total and conjugated bilirubin levels.

Similarly, Adedeji *et al.* (2005) reported the effect of different dietary inclusion levels of bitter kola on blood profiles of rats. The rats were placed on diets containing 0% (w/w), 5% (w/w), 10% (w/w) and 20% (w/w) levels of *Garcinia kola* for six weeks. It was observed that there was no significant difference between rats fed control diet and those in various dietary groups for all the blood parameters checked with the exception of the lymphocyte count

which had a significant difference in all dietary groups less than the control. According to Esiegwu and Udedibie (2009), there were no significant differences among the groups fed *Garcinia kola* in most of the haematological indices; however, the control group had more RBC than the *Garcinia kola* groups but significantly lower WBC.

Effect of Garcinia kola on organ characteristics

Uko *et al.* (2001) reported that water extract from *Garcinia kola* fed to rats did not significantly influence the organ of the control and experimental rats; however, there was a dose-related decrease in size of livers, lungs and hearts of rats fed the plant extract. The organs (testes, kidney, liver, heart, lungs and brain) did not show any microscopic alterations across treatment groups. It has also been reported by Braid (1989) that male rats fed diets containing 10% (w/w) dry powdered seeds of *Garcinia kola* for six weeks showed marked inhibition of gastro-intestinal motility, were protected against castor oil induced diarrhoea, exhibited prolonged pentobarbital sleeping time, marked retardation of growth but did not record organ weights effects compared to pair fed controls.

However, the report of Braid and Grill (1990) revealed some histological alterations in the liver, kidney and duodenum of rats fed diets containing 10% (w/w) dry powdered bitter kola for six weeks. The main cellular changes included vacuolation of duodenal villous epithelial cells, numerous intracytoplasmic vacuoles in hepatocytes and mild hydropic degeneration in cells of the renal proximal tubular epithelium. Contrary to the report of Braid (1989) and Uko *et al.* (2001), Esiegwu and Udedibie (2009) reported significantly heavier livers in *Garcinia kola* groups than the control group for broiler chicks placed on similar treatments for 8 weeks at 0, 2.5, 5.0, and 7.5% dietary levels.

Effect of *Garcinia kola* on reproduction

A Garcinia kola seed contain biflavonoid capable of having anti-inflammatory properties (Braid, 1993) and is a natural anti-oxidant (Olatunde *et al.*, 2002; Terashima *et al.*, 2002). The importance of the anti-inflammatory property of *Garcinia kola* is necessary because ovulation, an important process in female reproductive function, is believed to be an inflammatory process (Epsey, 1980; Epsey, 1994). Ovulation is brought about by a luteinising hormone (LH) surge. This surge of LH causes the follicle to rupture and hence ovulation. According to Gaytan *et al.* (2002), ovulation can be blocked experimentally by high doses of anti-inflammatory drugs administered before the LH surge because once the level starts to rise, it may not be stopped by any drug.

Akpantah *et al.* (2005) reported that *Garcinia kola* seed extract administered to rats at 200 mg/kg body weight altered oestrous cycle in rats, partly inhibited ovulation as evidenced by the reduced number of ova in the oviduct compared to the control, with a significant decrease in the weight of foetuses from the treated rats. Also 7% of the foetuses from pregnant rats which received treatment for the first five days of gestation had malformed left upper limb or morphological anormalies. It was also reported by Uko *et al.* (2001) that rats fed *Garcinia kola* seed extract exhibited increased libido (sexual instinct) for the male rats justifying the use of *Garcinia kola* by natives as an aphrodisiac, but did not improve pregnancy rates in female rats as a measure of the male fertility index.

The anti-inflammatory property of *Garcinia kola* seed may be responsible for the observed effect in blocking ovulation when administered to rats before the surge of luteinsing hormone (Freeman, 1988). The anti-inflammatory property of flavonoids is believed to result from inhibition of cyclo-oxygenase enzyme (Liang *et al.*, 1999). Akpantah *et al.* (2005) suggest that *Garcinia kola* seed may block ovulation by inhibiting cyclo-oxygenase activity and prostaglandin synthesis. Some flavonoids suppress the formation of cyclo-oxygenase – 2, thus playing an important role in the prevention of cancer and inflammation. This property has

been tried because of its chemo-prevention potentials against human cancers as many types of cancer cells use cyclo-oxygenase – 2 to propagate (Liang *et al.,* 1999). Similarly, cyclo-oxygenase – 2 (COX-2) deficient mice suffer from defect in reproductive functions such as ovulation and fertilization (Lim *et al.,* 1997).

Anti-microbial activities of *Garcinia kola*

Extracts from the bark, stem and seed of *Garcinia kola* have been reported to inhibit the growth of *plasmodium falciparum* by well over 60% *in vitro* at a concentration of 6 mg/ml (Tona *et al.,* 1999). The antimicrobial activities of aqueous extract of three Nigerian medicinal plants, *Vernonia amygdalina* (bitter leaf, BL), *Garcinia kola* (Bitter kola, BC) and *Gongronema latifolium* (Utazi, UT) and their blends were evaluated against several test organisms, *staphylococcus aureus, Bacillus subtilis*, *Escherichia coli* and *streptococcus salivanus* by Oshodi *et al.,* (2004). The study revealed that media containing a crude extract of UT showed no zone of growth inhibition against *Escherichia coli* and *Streptococcus salivanus,* while BC had no effect on *Escherichia coli* at all. UT: BL: BC and BL: BC blends were the most active blends while BL was the most active single plant extract.

A study by Akoachere *et al.* (2002) to investigate the anti-bacterial activity of bitter kola *(Garcinia kola)* and ginger *(Zingiber officianale)* on four respiratory tract pathogens, viz *Staphylococcus aureus, Streptococcus pyogens, Streptococcus pneumoniae and Haemophilus influenza* revealed that the extract from ginger and *Garcinia kola* exhibited anti-bacterial activity against the pathogens. A study by Elekwa (2003) on the effect of aqueous extracts of *Garcinia kola* seeds on membrane stability of human erythrocytes showed the possible use of the extract for the management of sickle cell crisis.

The seed of *Garcinia kola* has shown anti-inflammatory, anti-bacterial, anti-microbial, and anti-viral properties (Ebana *et al.,* 1991; Akoachere *et al.,* 2002). According to Esiegwu and Udedibie (2009), *Garcinia kola* seed meal fed to broiler chicks at 5.0 and 7.5% dietary levels suppressed the growth of *Salmonella species* in the birds but had no effect on *Escherichia coli.* In laboratory tests, bitter kola was found to halt the deadly disease caused by Ebola virus in its tracks, compounds from the plant have also proved effective against some strains of flu, a contagious respiratory disease also called influenza (Iwu, 1993). The seed of *Garcinia kola* has also shown a mild bronchodilator effect (Orie and Ekon, 1993). A study to investigate the anti-ulcerogenic and gastric acid lowering effects of *Garcinia kola* seeds in male albino rats containing 25%, 50% and 75% by weight of bitter kola showed a dose-dependent inhibition of gastric acid secretions and indomethacin-induced ulceration (Ibironke *et al.,* 1996).

Aniche and Uwakwe (1990), compared the chemical, brewing and anti-microbial properties of *Garcinia kola* with traditional hops and found that hops had higher concentration of organic acid than *Garcinia kola*. Laboratory brewing trials with *Garcinia kola* and hops gave beers with similar chemical properties, while organoleptically, *Garcinia kola* beer was as acceptable to tasters as hopped beer except that it had improved bitterness. Again, *Garcinia kola* and hop extracts exerted similar anti-microbial effects on two beer spoilage micro-organisms *(Lactobacillus delbruckii and Candida vini).*

Pharmacological mechanisms of *Garcinia kola* in animal models

The pharmacodynamic mechanism of *Garcinia kola* is anchored on kolaviron (Farombi *et al.,* 2000; Farombi, 2004; Adaramoye *et al.,* 2005), which is a yellow solid when extracted and is a mixture of Garcinia biflavonones GBI, GB2 and kola flavanone (KF). According to Iwu *et al.,* (1990), Kolaviron is a type of biflavonoids obtained from *Garcinia kola* seeds and produces significant hypoglycemic effects when administered intraperitoneally to normal and alloxan diabetic rabbits at a dose of 100 mg/kg. The fasting blood sugar in normoglycaemic

rabbits is reduced from 115 to 65 mg/100ml after 4 hours and in alloxan diabetic rabbits, while the blood sugar is lowered from 506 to 285 mg/ml after 12 hours (Iwu *et al.,* 1990).

Kolaviron, has also been shown to modulate the hepatoxicity of carbon tetrachloride, galactosamine, aminata toxin, paracetamol thioacetamide and 2-acetyl-aminofluorene in various experimental models. The hepatoprotective effect of the seed extract was investigated in rats treated with high doses of paracetamol. When administered at 100 mg/kg three times daily for 5 consecutive days the extract reduced paracetamol – (800, 1000, 1200 mg/kg), induced lethality from 50, 90, and 100% to 0, 20 and 40%, respectively (Akintowa and Essien, 1990).

In addition, the anti-hepatotoxic properties of *Garcinia kola* have been evaluated using four experimental toxins, namely carbon tetrachloride, galactosamine, alpha amanitin and phalloidin. Kolaviron, a fraction of the defatted ethanol extract and two billabongs of *Garcinia kola* seed (GB1 and GB2), significantly modified the action of this hepatitis. At 100mg/kg orally, the test substances reduced thiopental-induced sleep in CC14 poison rats and protected microsomal enzymes against phalloidin toxin (Iwu *et al.,* 1987). Kolaviron from *Garcinia kola* seeds reduced lethal poisoning of mice by phalloidin. The biflavanones GB1, GB2 and kolaflavanone were isolated as the active constituents (Iwu, 1985).

Kolaviron, has also been shown to prevent lipid peroxidation products and protect biomembranes against oxidative damage by acting as *in vivo* anti-oxidant in animal studies (Farombi *et al.,* 2000). *Garcinia kola* inhibited *in vitro* lipid peroxidation of rat liver homogenate in a dose-dependent manner (Adegoke *et al.,* 1998). Possible anti-atherogenic effects of kolaviron (a *Garcinia kola* seed extract) in hypercholesterolaemic rats were investigated and it was revealed that kolaviron reduced plasma cholesterol levels and the relative weight of the heart in cholesterol fed animals (Adaramoye *et al.,* 2005).

Garcinia kola clinically appears to have a significant analgesic/anti-inflammatory effects in knee osteoarthritis patients. According to Olayinka *et al.* (2008), it is effective in improving locomotors function and significant pain reduction in patients with knee osteoarthritis. Again, extracts from the seeds of *Garcinia kola* have been shown to exhibit excellent bactericidal properties (Okwu, 2004).

Safety of *Garcinia kola*

Garcinia kola is safe consumed with or without other foods. Its consumption an hour before or after meals may help to increase the absorption of key ingredients.

Food does not affect the metabolism of *Garcinia kola* and may suffer the effects of mild indigestion (Iwu, 1986). Kolaviron does not appear to have a pronounced effect on drug metabolizing enzymes (Farombi *et al.,* 2000) and no known interaction with orthodox medications has been reported (Okoli, 1991).

CONCLUSION

This review has shown that bitter kola has enormous health benefits that could be explored by adding it to animal feeds. It has shown that *Garcinia kola* is not deleterious to the health of animals. It has however, indicated that few studies have been carried out on the nutrient and phytochemical compositions of *Garcinia kola*.

Further studies are needed to fully understand its nutritional mechanisms in animal feeding and how it could best be utilized for this purpose.

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Original Article

Growth Performance and Carcass Characteristics of Horro Rams under Different Management Practices at Ambo University, Ethiopia

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ABSTRACT

This study was conducted to evaluate growth performance, carcass and non carcass characteristics of Horro Rams under different management practices at Ambo University. A total of 24 Horro rams were randomly assigned to the following three treatments: T1= Day 1 rest before slaughter (Animals slaughtered after transportation to experimental site), T2= Rhodes hay *ad libitum* and T3= Rhodes hay *ad libitum* + 400 g concentrate head/day. The initial, fortnight and slaughter live body weight were taken at the initial, fortnightly and at the end of the feeding trial. Average daily gain (ADG) was calculated as change in live body weight over total duration of fattening period. All the carcass and non carcass components were taken and recorded. Data were analyzed using the General linear model procedures of Statistical Analysis System Software 9.2. ADG of concentrate supplemented Horro rams (117.36 g) was greater ($P < 0.001$) than animals fed Rhodes hay *ad libitum (*11.11 g). Average hot carcass weight of supplemented animals (13.5 kg) was heavier ($P < 0.001$) than animals fed on Rhodes hay *ad libitum* and slaughtered after day one rest of transportation (8.4 and 8.93 kg, respectively). Concentrate supplementation had significant and positive influence on ADG, carcass and non carcass components of Horro rams. Therefore, management practices like optimum feeding would improve the growth performance, carcass and non carcass characteristics of Horro sheep.
Keywords: ADG, carcass, concentrate and Horro rams

INTRODUCTION

In Ethiopia, sheep population is ~25.5 million **(**CSA, 2013) and mainly kept for meat production (Ewnetu *et al*.. 2006). The annual mutton production of the country is estimated at 78 thousand metric tons and the annual average off-take rate for sheep is estimated to be about 35 % with an average carcass weight of about 10kg, which is the second lowest amongst sub -Saharan Africa countries (FAO, 2001). Despite the fact that huge genetic

diversity does exist in Ethiopia, profitability from sheep farming is limited by low performance in terms of market weight, reproductive efficiency and meat yield. Over all there is low off-take rate and very low meat yield, which is aggravated by poor feeding and management problems (Mukasa and Lahlou, 1995). Majority of the animals depend upon natural pasture besides crop residues to meet nutritional requirements, it has been observed that the quality and quantity of the pasture fluctuates seasonally thereby affecting the growth, carcass quality and quantity of the animals. Supplementation is very vital to improve the growth performance and mutton yield of local breeds.

Ethiopia has been exporting meat, live animals and skin to Middle East countries. There is a high demand of meat and live animals for domestic and export markets. It is an urgent need to improve the productivity of animals to meet high demand for livestock products. There is also a great need to meet the export standard of carcass and high demand of meat. Thus, the objective of this study was to evaluate growth performance, carcass and non carcass characteristics of Horro Rams under different management practices at Ambo University, Ethiopia.

MATERIAL AND METHODS

Location and facilities of experiment site

The study was conducted at Ambo University farm, which is approximately 115 km west of Addis Ababa, Ethiopia. Slaughter and associated measurements were performed at facilities of Animal Sciences Laboratory of the Ambo University. Rest and feeding of animals took place at a sheep feedlot facility (4 pens of 4 × 3.5 m) of the University.

Experimental Animals and treatments

Horro sheep are the long fat tailed highland sheep mainly found in Horro Guduru zone of western Ethiopia. Based on dentition, all animals were approximately 1 year of age and similar in average initial body weight (21.31 ± 2.16 kg) and conformation. Horro rams were purchased from 'Gabaa Sanbataa' market found in Horro district of the Horro Guduru zone, approximately 190 km from Ambo University. Animals were transported as procedures for procurement, transportation, and handling were the ones used by abattoirs in Ethiopia. A total of 24 Horro rams were randomly assigned to the following three treatments with 8 replicates:

T1= Day 1 rest before slaughter (Animals slaughtered after transportation to experiment site)
T2= Rhodes hay *ad libitum* or control group
T3= Rhodes hay *ad libitum* + 400 g concentrate per day/ head of animal

Those animals randomly assigned for 90 days fattening treatments were drenched with albendazole and sprayed with diazinon. There was no health problem encountered during the experiment period. The concentrate composition is 49.5% noug cake (Gizotia abyssinica), 49.5 % ground maize grain and 1% salt. Water and moderate quality grass hay were provided *ad libitum* for all treatments until slaughter until approximately 12:00 h the day preceding slaughter.

Measurements

The initial, fortnight and slaughter live body weight were taken at the initial, fortnightly and at the end of the feeding trial. Average daily gain (ADG) was calculated as change in live body weight over total duration of fattening period. The experimental animals were slaughtered after one day rest of arrival at experimental site and at the end of 3 months

fattening period. All the carcass and non carcass components were taken and recorded during the slaughtering time. Empty body weight was estimated as the sum of the carcass and non-carcass components with digests excluded.

Statistical Analysis

Data were analyzed using the General linear model procedures of Statistical Analysis System Software 9.2 (SAS, 2008). During analysis, treatment was considered as independent variable whereas average daily weight gain, carcass and non carcass components considered as dependent variables. Initial body weight (BW) was used as a covariate for ADG. Means were separated by least significant difference.

RESULTS AND DISCUSSION

Growth Performance of Horro rams

Concentrate supplementation had significant effect on average daily weight gain (ADG) and as expected, the ADG was greater ($P < 0.001$) for concentrate supplemented Horro rams compared to animals fed Rhodes hay *ad libitum* (Table 1).

Table 1: Least square means of live body weights and average daily gain (ADG) of Horro rams under different management practices

TRT	Initial Body weight (kg)	Final Body weight (kg)	ADG (g/day)
1	21.63	-	-
2	21.64	22.64[b]	11.11[b]
3	21.45	32.00[a]	117.36[a]
SE	2.16	2.49	24.21
CV	-	9.03	15.72
R^2	-	80.12	84.69
P- value	-	0.0001	0.0001

T1: Animals slaughtered after day 1 rest of transportation; T2: Rhodes hay *ad libitum* and T3: Rhodes hay *ad libitum + 200g noug cake + 200g ground maize grain per day/head of animal.*
[ab] Means within rows without common superscript differ significantly at $P < 0.001$.

The change in live body weight/growth curve was substantially increased up to 30 days fattening duration and then after increased slightly which could be explained as compensatory growth in the first days of fattening periods (Figure 1).

The ADG of concentrate supplemented Horro rams obtained in this experiment (117.36 g/day) was similar to the earlier report of ADG by Galal *et al.*. (1981), who reported 118 g/day for 12 months Horro sheep supplemented on 400 g concentrate/head/day after daytime grazing at Bako Agricultural Research Center but greater than the ADG reported by Ewnetu *et al.*. (2006) and Kassahun (2000), who reported 47.3 and 70.9 g/day per animal for Horro male lambs, respectively. In line with these results, Chala *et al.* (2013) reported that daily live weight gains were greater ($P < 0.05$) for Horro ewe lambs supplemented with Leucaena pallida and concentrate compared with the un-supplemented control treatment. Santos-Silva *et al.*. (2004) also reported that hay fed lambs showed lower intake, average daily weight gain and slaughter weights than those fed pellets.

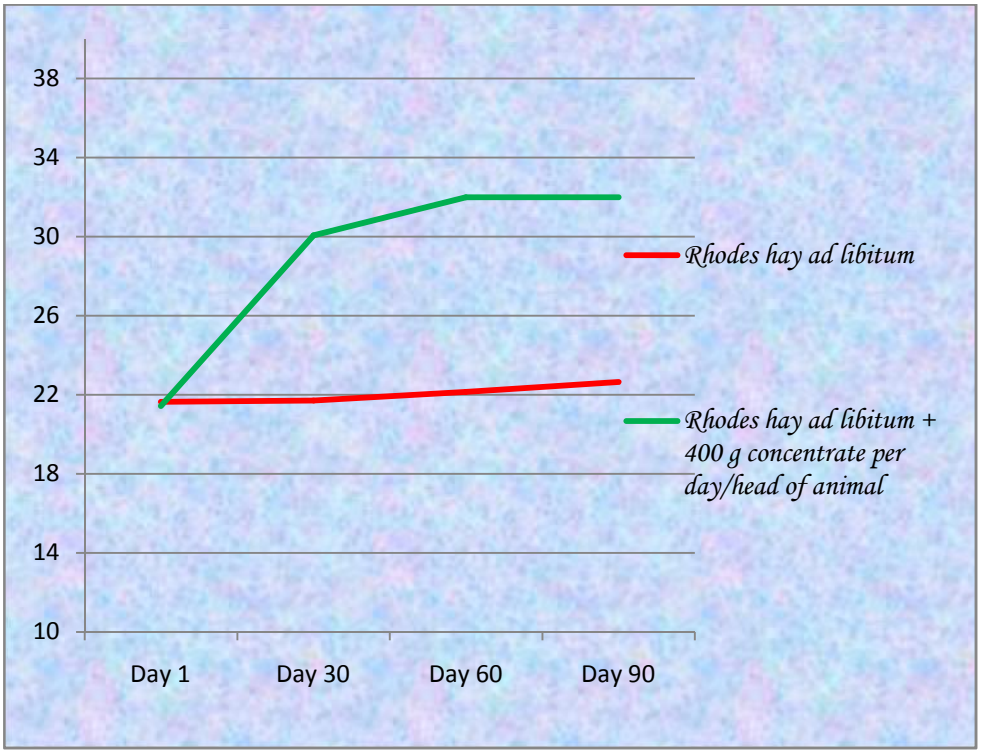


Figure 1: Change in live body weight (growth curve) of Horro rams under different management practices during fattening period

Carcass and non carcass components

Concentrate supplementation had significant positive influence on carcass weight, dressing percentage, tail fat weight and kidney with fat of Horro rams (Table 2; Picture 1). Hot carcass weight was heavier ($P < 0.001$) for supplemented animals compared to animals fed on Rhodes hay *ad libitum* and slaughtered immediately after day 1 rest of arrival/transportation to experiment site. Dressing percentages on slaughter and empty body weight basis, tail fat weight and kidney with fat of supplemented Horro rams were greater ($P < 0.05$) than other treatment animals.

Table 2: Least square mean values of carcass components of Horro Rams under different management practices at Ambo University

Carcass components	Treatments			Overall mean	SE	CV	R^2	P-value
	1	2	3					
Hot carcass weight (kg)	8.40 [b]	8.93 [b]	13.50 [a]	10.34	1.15	11.11	63.58	0.0001
Dressing % on Slaughter body weight basis	38.88 [b]	39.28 [b]	42.24 [a]	40.17	2.51	6.25	29.70	0.0295
Dressing % on empty body weight basis	45.54 [b]	46.19 [b]	48.39 [a]	49.89	3.29	7.04	14.09	0.0219
Kidney with fat (g)	81.63 [b]	104.29 [b]	173.25 [a]	120.39	18.40	15.29	84.24	0.0001
Heart (g)	105.5	104.72	287.38	168.52	219.99	13.49	15.19	0.1924
Liver (g)	331.38	371.86	422.00	375.22	111.49	29.72	11.71	0.2897
Tail weight	312.5 [b]	347.29 [b]	1063.3 [a]	584.22	331.48	56.74	56.29	0.0003

T1: Animals slaughtered after day 1 rest of transportation; T2: Rhodes hay *ad libitum* and T3: Rhodes hay *ad libitum + 200g noug cake + 200g ground maize grain per day/head of animal.*

[ab] Means within rows without common superscript differ significantly at $P < 0.001$.

In agreement to these results, Chala *et al.* (2013) reported that Horro rams supplemented with 400g concentrate and cyndon dactyl hay ad libitum had greater carcass weight (11.6 kg) and dressing percentage (45.2%). Ulfina *et al.* (2004) also reported that there was improvement in carcass weight and dressing percentage with increased level of concentrate supplementation. Merera *et al.* (2009) concluded that 2 weeks feeding period could be employed with highland sheep to markedly increase carcass weight. Similarly, earlier report

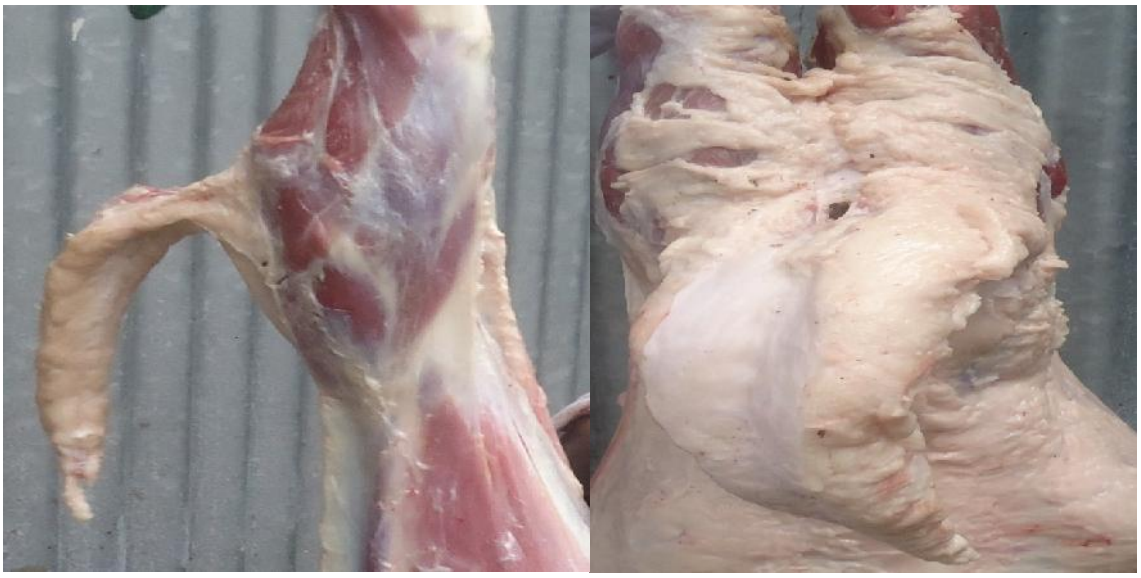
by Galal *et al.* (1981) on the same sheep breed showed significant differences between realimented and continuously fed lambs in carcass weight and dressing percentage and fat deposition measurements. In accordance with the above results, Priolo *et al.* (2002) also revealed that carcasses from stall-fed lambs were heavier than those from grass-fed lambs and carcasses from stall lambs had better muscular conformation score ($P < 0.05$) and were fattier than those from grass-fed animals.

Supplementation had also improved the mass weight of skin, omental fat, spleen, head and the visceral full ($P < 0.01$; Table 3). The greater yield of skin is practically useful for leather industry or export market. Likewise, higher mass of non-carcass tissues available for market has important for domestic consumption and export. Similar mass weights of non carcass components were reported by different researchers (Chala *et al..*, 2013; Ulfina *et al..*, 2004 and Galal *et al..*, 1981).

Table 3: Least square mean values of non carcass components of Horro Rams under different management practices

Non Carcass components	Treatments			Overall mean	SE	R^2	CV	P-value
	1	2	3					
Skin (kg)	1.99 [b]	2.87 [a]	3.06 [a]	2.59	0.50	55.5	19.3	0.0003
Visceral full (kg)	3.11 [b]	3.35 [b]	4.00 [a]	3.49	0.48	41.8	13.8	0.0004
Head (g)	1325.0 [b]	1225.7 [b]	1504.8 [a]	1357.3	169.9	34.4	12.5	0.0147
Legs (g)	448.9 [a]	515.9 [b]	628.4 [c]	531.7	60.3	64.4	11.3	0.0001
Lung and track (g)	261.8 [a]	330.7 [b]	430.3 [c]	341.4	49.4	70.2	14.5	0.0001
Omental fat (g)	33.5 [b]	53.5 [b]	129.0 [a]	79.9	37.9	59.6	17.5	0.0018
Spleen (g)	39.8 [a]	36.9 [a]	57.0 [b]	44.9	10.5	45.6	23.3	0.0023
Blood (g)	1136.3	966.4	1100.4	1072.1	293.1	6.5	27.3	0.5162
Testicles (g)	268.3	261.4	951.8	503.9	875.9	13.8	13.8	0.2261

T1: Animals slaughtered after day 1 rest of transportation; T2: Rhodes hay *ad libitum* and T3: Rhodes hay *ad libitum + 200g noug cake + 200g ground maize grain per day/head of animal.*
[abc] Means within rows without common superscript differ significantly at $P < 0.001$.



Picture 1: Hot carcass weight, conformation and composition of Horro rams fed up on Rhodes hay *ad libitum* (left side) and 400 g concentrate head/day +Rhodes hay *ad libitum (right side) at Ambo University*

CONCLUSION AND RECOMMENDATION

The study was conducted to evaluate growth performance, carcass and non carcass characteristics of Horro Rams under different management practices at Ambo University. ADG of concentrate supplemented Horro rams (117.36 g) was greater ($P < 0.001$) than animals fed Rhodes hay *adli bitum (*11.11 g). Average hot carcass weight of supplemented animals (13.5 kg) was heavier ($P < 0.001$) than animals fed on Rhodes hay *ad libitum* and slaughtered after day one rest of transportation (8.4 and 8.93 kg, respectively). Concentrate supplementation had significant and positive influence on ADG, carcass and non carcass components of Horro rams. Therefore, management practices like optimum feeding would improve the ADG and yield of mutton.

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