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EFFECT OF LONG TERM DIETARY SUPPLEMENTATION OF *LIPPIA CITRIODORA* EXTRACT ON SEMEN QUALITY TRAITS IN BROWN HARE (*LEPUS EUROPAEUS*)

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ABSTRACT

There is an internationally growing interest concerning application of natural extract sources in animal production area in order to improve the husbandry welfare and the performance. The aim of the present study was to evaluate the effect of dietary supplementation with a natural extract of *Lippia citriodora*, containing verbascoside as main component, on some quality traits of semen of hare, monitoring also the welfare status of animals.
Hares were randomly divided into four groups of 3 animals each, homogeneous by age and body weight, and fed *ad libitum* and free access to water until the end of the trial. Animals were fed for 240 days a commercial diet assigned to four dietary treatments: control diet (CON) and the diet supplemented with 1 g.kg^{-1} of natural extract (low natural extract – LNE) or 1.5 g kg^{-1} of natural extract (medium natural extract – MNE) or 2 g.kg^{-1} of natural extract (high natural extract – HNE). All hares were subjected to the following experimental measurements: weekly relief of feed intake, body weight and blood samples at 0 and at 240 day of trial, and semen collection at 180, 210 and 240 days of trial.
The body weight of the hares and their feed intake were not affected by the experimental treatment. At the end of the trial, sperm volume, pH and sperm concentration values were not effected by *Lippia citriodora* extract treatment, and the mean values recorded were 0.543 ml, 7.4 and 263.25 10^6 per ejaculate, respectively. The dietary treatment negatively affected ($P < 0.05$) the sperm motility values in LNE, MNE and HNE groups.
In conclusion, the results of the present work underline a possible negative effect of the *Lippia citriodora* extract on the semen quality characteristics, besides the improvement in welfare status of the treated hares, reflected in a better lipid profile and an improved plasma oxidative markers.

Key words: antioxidant supplement; biochemical parameters; hare spermatozoa

INTRODUCTION

There is an internationally growing interest concerning application of natural extract sources in animal production area in order to improve the husbandry welfare and the performance. Natural antioxidants have been widely reported to have potent antioxidant, anti-inflammatory and antimicrobic activities related especially to their phenolic content (Pereira *et al*., 2009).

Lippia citriodora, a plant species in the Verbenaceae family, is characterised by the presence of several phenolic compounds, including flavonoids, phenolic acids, luteolin derivatives and phenylpropanoids (Valentão *et al*., 2002) that are the most abundant compounds in Verbenaceae extracts (Pascual *et al*., 2001). Phenylpropanoid glycosides are powerful antioxidants acting by direct scavenging of reactive oxygen and nitrogen species, or as chain-breaking peroxyl radical

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scavengers (Afanasev, 2005).

Previous studies (Palazzo *et al.*, 2011; Casamassima *et al*., 2012, 2015) showed that dietary supplementation with *Lippia* extracts in different species such as hare, sheep and rabbit showed an improvement in biochemical parameters, characterised by a decrease in the concentration of triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol, bilirubin, reactive oxygen metabolites (ROM) and thiobarbituric acid reactive substances (TBARS), by an increase in high density lipoprotein (HDL) cholesterol and plasma vitamin A and E.

Therefore, the aim of the present study was to evaluate the effect of dietary supplementation with a natural extract of *Lippia citriodora*, titrated in verbascoside, on some quality traits of hare semen, monitoring also the welfare status of animals.

MATERIAL AND METHODS

The experiment lasted 240 days and was performed on the farm "Allevamenti Roger" in the countryside of Isernia (Molise region, Italy) on 12 fertile and healthy hare males (*Lepus europaeus* Pallas, 1778) housed individually. All the breeding procedures and management of animals were conducted in accordance with the European Directive 2010/65/ EU regarding the protection of animals used for scientific purposes.

To ensure that animals adapt to the experimental condition, an adaptation period of 30 days was kept; after that hares were randomly divided into four groups of 3 animals each, homogeneous by age (270 ± 5 days of age) and body weight (2.8 ± 0.2 kg), and fed *ad libitum* with free access to water until the end of the trial (510 day of age). Animals were fed a commercial diet assigned to four dietary treatments: control diet (CON) and diet supplemented with 1 $g.kg^{-1}$ of natural extract (low natural extract - LNE) or 1.5 $g.kg^{-1}$ of natural extract (medium natural extract - MNE) or 2 $g.kg^{-1}$ of natural extract (high natural extract - HNE). The chemical composition of the feed (AOAC, 2000) was following (per kg of dry matter): crude protein 154 g; crude fat 33 g; crude fiber 195 g; Neutral Detergent Fiber 385 g; Acid Detergent Fiber 240 g; ashes 85 g, moisture 111 g. The experimental diets were prepared by adding the natural extract to the basal commercial mashed diet (4 mm pellets). Hares also had *ad libitum* access to alfalfa hay.

The antioxidant supplement contains a water-soluble extract of *Lippia citriodora* leaves (Verbenaceae, *Lippia* NE), prepared on an industrial basis by a standardised procedure that includes ultrasonic extraction with 60 % aqueous ethyl alcohol followed by spray drying with maltodextrins as an excipient. The bioactive components of the feed supplement, according to a certificate of analysis provided by the manufacturer were: verbascoside 4.47 ± 0.08, methyl gallate 1.91 ± 0.09, gallic acid 1.75 ± 0.07, 3.4-dihydroxybenzoic acid 0.45 ± 0.04 and isoverbascoside 0.43 ± 0.04 $g.kg^{-1}$.

The quantitative analysis of the phenolic compounds was performed by HPLC-UV-DAD according to Piccinelli *et al.* (2004). To avoid oxidation in the complete feed, the supplement is micro-encapsulated within a protective matrix of hydrogenated vegetable lipids using spray-cooling technology (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy).

All hares were subjected to the following experimental measurements: weekly relief of feed intake, body weight and blood samples at 0 and 240 days of trial, and semen collection at 180, 210 and 240 days of trial.

Blood samples were collected in the morning from the external ear vein by immobilizing the animal in a tissue bag, from which only the ears protruded through the slots. The bag, made to fit the animal, maintained their stillness with darkness to keep them calm. Triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin levels in plasma were immediately determined with an automated clinical chemistry analyzer, model ARCO (Biotecnica Instruments S.p.A., Roma, Italy). The concentration of ROMs in plasma was determined by a spectrophotometer and a colorimetric method, as proposed by Diacron, using a specific commercial kit at a wavelength of 505 nm (Cesarone *et al*., 1999). The results were expressed in Carr Units (1 Carr Unit equals 0.024 $mmol.l^{-1}$ of H_2O_2). The determination of TBARS was performed in plasma according to Esterbauer and Zollner (1989). The results were expressed as mmol of thiobarbituric acid per litre of plasma. Vitamins A and E were extracted from plasma samples with chloroform (Zhao *et al*., 2004) and analyzed on an HPLC system (Kontron Instruments, Milano, Italy).

Semen samples were collected with electroejaculation method according to Kozdrowski and Dubiel (2005), giving intramuscularly medetomidine (Domitor®) at dose of 0.1 $ml.kg^{-1}$ body weight. After few minutes from achieving full anesthesia, 4 ml of warm physiological saline were infused to the rectum and rectal probe was inserted into rectum as deep as was possible. Semen collection followed the commonly accepted rules, and after four to five impulses the ejaculated liquid was sampled. There were no adverse effects after electroejaculation and the interval between consecutive semen collections was four weeks. Following semen collection the volume of ejaculate was recorded and the percentage of motile spermatozoa was assessed

in light microscope (Alphaphot-2 YS2, Nikon, Tokyo, Japan) equipped with a thermostable table of 37 °C, under 200 x magnification. The concentration of spermatozoa in a whole ejaculate was assessed using Spermacue® photometer (Minitube®, Bio-One GmbH, Germany) and the pH value of spermatic liquid was assessed with a Hanna Instrument pH-meter (Woonsocket, USA) with a specific probe. For all experimental determinations each sample was analyzed in triplicates.

Statistical analysis

After verifying the normality of the frequency distribution, all variables were subjected to a variance test using the GLM procedure for repeated measures in the SPSS program (2010). The fixed effects of dietary treatment and time, as well as their interaction, were included in the model. Differences were considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

During the whole test period, the welfare status of animal was always considered to be good. The present study was not focused on growth performance of hares since a low number of replicates were used.

The body weight and feed intake of the hares were not affected by the experimental treatment. In general, any effect from the addition of natural extract should not be expected, when animals are healthy and housed in a clean environment. Moreover, the animals used in this experiment were adult.

The dietary treatment with *Lippia citriodora* extract affected ($P < 0.05$) all tested serum biochemical parameters, excepting AST and ALT values (Table 1).

In the three experimental groups, the triglycerides ($P < 0.01$) at the end of the trial were lower than in the CON group. The time affected the triglyceride values; in fact, from the beginning to the end of the test they decreased significantly in the LNE, MNE and HNE groups, whereas in the same period CON-group values were unchanged.

Total cholesterol was influenced by the dietary treatment at the end of the trial, showing a significant decrease in the MNE and HNE groups compared with the CON group. Time effect on total cholesterol values showed a decrease in the MNE and HNE groups, whereas in the CON and LNE group total cholesterol values were unchanged.

The HDL cholesterol increased significantly at 240 d of sampling in LNE, MNE and HNE groups compared to the CON group. A time effect was also observed, from the beginning to the end of the trial. Serum HDL cholesterol was statistically higher in the LNE, MNE and HNE groups, whereas values remained almost unchanged in the CON group.

The LDL cholesterol values were significantly lower in the LNE and HNE groups compared with the CON group at the end of the trial. A time effect was observed during the whole trial on that parameter, decreasing in the LNE, MNE and HNE groups.

The better lipid profile, recorded in the present experimental trial, with the use of *Lippia* NE, through the increase in plasma concentration of HDL cholesterol, may be due to the effect of polyphenols, which are involved in the regulation of lipid and glucose metabolism. According to some authors (Norata *et al*., 2003; Bursill and Roach, 2007), this activates the PPAR-α receptor, with an increased stimulation effect in the liver of the expression of key proteins involved in the metabolism of HDL. Triglycerides would also seem to be involved in the same mechanism of activation of PPAR-α by the polyphenols, with an induction in lipoprotein lipase expression in peripheral tissues and increased lipolysis, which probably results in a reduction in circulating triglycerides and very low density lipoprotein.

Our previous experiments in sheep, hare and rabbit, fed with a dietary NE supplement, revealed a significant reduction in triglycerides, total cholesterol and LDL cholesterol along with increased HDL cholesterol (Palazzo *et al*. 2011; Casamassima *et al*. 2012, 2015). In broilers fed the diet enriched with thyme leaves, Radwan (2003) and Case *et al*. (1995) found a reduction in total lipids and total cholesterol. This was probably due to an inhibiting effect of the enzyme HMG-CoA reductase by thymol and carvacrol, which are responsible for the cholesterol synthesis in the liver.

Serum AST and ALT showed a tendency to decrease in all three experimental groups, with no statistical significance, compared to the CON group. Moreover, values of AST and ALT remained within the normality range of the species, and no hepato-toxicity to animals was found.

Serum values of bilirubin decreased ($P < 0.05$) in the LNE, MNE and HNE groups at the end of the trial due to the dietary treatment. The decrease in bilirubin could be attributed to the antioxidant activity of polyphenols, which inhibits the biochemical mechanisms involved in the formation of the same bilirubin (Aliyu *et al*., 2007).

Table 2 reported data on the plasma oxidative markers in hares fed *Lippia* NE extract.

The ROM values markedly decreased in the LNE, MNE and HNE groups compared to the CON. The duration of treatment resulted in a significant decrease in ROM values in all three experimental groups, whereas in the CON group the concentration during the same period of time remained unchanged.

The TBARS markedly decreased in the treated groups compared with the CON, with a decrease in LNE,

Table 1: Biochemical parameters in hares fed *Lippia citriodora* extract

Parameters	Experimental groups§				SEM¶	P-value‡		
	CON	LNE	MNE	HNE		D	T	D×T
Animals (n)	3	3	3	3				
Triglycerides (mg.dl^{-1})								
0 d	120.7	118.7^{a}	121.7^{a}	118.3^{a}	3.13			
240 d	119.0^{1}	111.0^{2b}	112.0^{2b}	102.2^{3c}	7.18	0.008	0.009	0.049
Total cholesterol (mg.dl^{-1})								
0 d	25.6	23.8	26.0^{a}	24.2^{a}	2.41			
240 d	25.9^{1}	22.4^{12}	19.7^{2b}	20.7^{2b}	3.67	0.225	0.039	0.049
HDL cholesterol (mg.dl^{-1})								
0 d	5.1	5.2^{a}	5.6^{a}	5.5^{a}	1.54			
240 d	4.9^{1}	6.1^{2b}	7.1^{3b}	6.9^{3b}	1.20	0.049	0.048	0.033
LDL cholesterol (mg.dl^{-1})								
0 d	8.1	8.2^{a}	8.2^{a}	8.1^{a}	0.66			
240 d	8.2^{1}	7.1^{2b}	7.2^{2b}	6.6^{2b}	1.05	0.045	0.045	0.038
AST (UI)								
0 d	108.0	107.8	107.7	107.6	2.93			
240 d	111.1	107.5	107.4	107.3	3.84	0.348	0.489	0.445
ALT (UI)								
0 d	56.4	55.2	57.7	58.0	3.19			
240 d	57.3	52.9	54.3	54.9	3.54	0.053	0.125	0.231
Bilirubin (mg.dl^{-1})								
0 d	0.60	0.62^{a}	0.63^{a}	0.60^{a}	0.07			
240 d	0.61^{1}	0.53^{2b}	0.53^{2b}	0.47^{3b}	0.08	0.042	0.015	0.048

§ Control (CON); 1 g.kg^{-1} low natural extract (LNE); 1.5 g.kg^{-1} medium natural extract (MNE); 2 g.kg^{-1} high natural extract (HNE).
¶ SEM: Standard error of means
‡ D: fixed effect of dietary supplementation; T: fixed effect of time; D×T: interaction dietary supplementation x time.
^{1,2,3} Within a row, means without a common superscript differ ($P < 0.05$).
^{a,b} Within a column, means without a common superscript differ ($P < 0.05$).

MNE and HNE groups. The duration of treatment also resulted in a significant decrease in the LNE, MNE and HNE group values, whereas the CON group showed a tendency to increase of TBARS. The experimental treatment led to an improvement in the markers of plasma oxidative status. As a redox-active molecules (capable of being oxidised and reduced without becoming a highly reactive-radical molecule), the NE protects against ROS, with a consequent reduction in lipid peroxidation, as also highlighted by the decrease in plasma levels of TBARS. The reduction in ROM and lipid peroxidation could be attributed both to the direct capture of free radicals due to the antioxidant activity of NE during the propagation phase of the chain reaction, and to a block of the initial oxidative process, through the inhibition of the pro-oxidant enzymes that produce free radicals (Kamiloglu *et al.*, 2006). In our previous research (Palazzo *et al*., 2011; Casamassima *et al*., 2012), we also found an improvement in the markers of plasma oxidative status, in sheep and hare fed *Lippia* NE supplemented in the diet.

In the HNE, MNE and LNE groups, a marked increase in vitamin E concentration in blood plasma samples was observed compared to the CON group. The duration of treatment influenced the concentration of vitamin E; in fact from the beginning to the end of the trial it was increased significantly in the three experimental groups, whereas in the CON group concentrations remained unchanged.

Table 2: Plasma oxidative markers in hares fed *Lippia citriodora* extract

	Experimental groups§					P-value‡		
Parameters	CON	LNE	MNE	HNE	SEM¶	D	T	D×T
Animals (n)	3	3	3	3				
ROMs (U.Carr^{-1})								
0 d	187.7	190.7^{a}	186.5^{a}	186.5^{a}	9.46			
240 d	199.9^{1}	137.1^{2b}	110.2^{3b}	122.4^{2b}	9.39	0.049	0.042	0.036
TBARS (mmol.L^{-1})								
0 d	0.162	0.152^{a}	0.166^{a}	0.171^{a}	0.034			
240 d	0.222^{1}	0.126^{2b}	0.128^{2b}	0.128^{2b}	0.048	0.004	0.042	0.045
Vitamin E (micr-mol.L^{-1})								
0 d	0.315	0.326^{a}	0.317^{a}	0.326^{a}	0.023			
240 d	0.299^{1}	0.367^{2b}	0.389^{2b}	0.455^{3b}	0.069	0.003	0.002	0.039
Vitamin A (micr-mol.L^{-1})								
0 d	0.252	0.267^{a}	0.278	0.275^{a}	0.017			
240 d	0.247^{1}	0.286^{2b}	0.285^{2}	0.332^{3b}	0.016	0.048	0.041	0.045

§ Control (CON); 1 g.kg^{-1} low natural extract (LNE); 1.5 g.kg^{-1} medium natural extract (MNE); 2 g.kg^{-1} high natural extract (HNE).
¶ SEM: Standard error of means
‡ D: fixed effect of dietary supplementation; T: fixed effect of time; D×T: interaction dietary supplementation x time.
^{1,2,3} Within a row, means without a common superscript differ ($P < 0.05$).
^{a,b} Within a column, means without a common superscript differ ($P < 0.05$).

The LNE, MNE and HNE groups showed a significant increase in the plasma concentration of vitamin A in LNE, MNE and HNE group, compared to the CON group. From the beginning to the end of the trial these values significantly increased in LNE and HNE groups, whereas in the CON and MNE group concentration remained unchanged. The increase in plasma vitamin A and E may be attributed to the ability of the NE to strengthen the endogenous antioxidant system. This is achieved by controlling the oxidative metabolism by reducing the production of reactive oxygen radicals and by inducing enzymes with antioxidant activities (Zhu *et al.,* 1999). Similar results were obtained in our previous studies on hare, on naturally milk-fed lambs and on ewes whose diet was supplemented with *Lippia* NE (Palazzo *et al.*, 2011; Casamassima *et al*., 2012, 2013a, 2013b).

Table 3 reported data on semen quality characteristics of hares, taken at 180 d, 210 d and 240 d.

All recorded data remained within the normality range of the species (Kozdrowski and Dubiel, 2005) and only the sperm motility values were affected by the dietary treatment.

At the end of the trial, sperm volume, pH and sperm concentration values were not affected by *Lippia* NE treatment, and the mean values recorded were 0.543 ml, 7.4 and 263.25 10^{6} per ejaculate, respectively.

The dietary treatment negatively affected ($P < 0.05$) the sperm motility values in LNE, MNE and HNE groups, by 21.0 %, 19.7 % and 18.9 %, respectively. A time effect was observed in CON group with an increase of values by 9.9 %, and in HNE group, with a decrease by 14.9 % at the end of the experiment.

These results are in agreement with those of Vizzarri *et al*. (2010), where a possible negative effect of verbascoside (main bio-active component of *Lippia* NE) in rabbit quality semen was reported. In addition Dell'Aquila *et al*. (2014) observed a pro-oxidant effect of verbascoside on ovine prepubertal oocytes in *in vitro* experiment.

Unexpectedly, the dietary use of antioxidant supplement did not provide any improvement to the semen quality traits, as generally reported in literature (Yousef *et al.*, 2003), but it improved the welfare status of treated animals, as was reflected in a better lipid profile and oxidative markers.

A negative correlation coefficients were also reported between semen quality characteristics and AST and ALT enzyme activities (Yousef *et al*., 2003), but in our experiment no such correlation was found.

Table 3: Semen quality characteristics in hares fed *Lippia citriodora* extract

Parameters	Experimental groups§				SEM¶	P-value‡		
	CON	LNE	MNE	HNE		D	T	D×T
Animals (n)	3	3	3	3				
Sperm volume (ml)								
180 d	0.450	0.417	0.410	0.457[a]	0.017			
210 d	0.453	0.360	0.443	0.463[a]	0.024			
240 d	0.517	0.523	0.467	0.667[b]	0.036	0.470	0.001	0.076
pH								
180 d	7.27[1]	7.03[1]	8.13[2]	7.40	0.143			
210 d	7.40	7.10	8.10	7.43	0.207			
240 d	7.47	7.23	7.83	7.17	0.121	0.096	0.860	0.837
Sperm concentration (n 10^6/ejaculate)								
180 d	261.00	257.00	251.00	273.67	3.437			
210 d	264.00	261.33	242.67[1]	279.33[2]	7.693			
240 d	265.33	264.67	245.00	278.00	5.141	0.055	0.741	0.679
Sperm motility (%)								
180 d	70.67[a]	71.33	72.33	74.00[a]	0.883			
210 d	69.00[a]	62.33	61.00	61.33[b]	1.438			
240 d	77.67[1b]	61.33[2]	62.33[2]	63.00[2b]	2.169	0.028	0.001	0.001

§ Control (CON); 1 g.kg^{-1} low natural extract (LNE); 1.5 g.kg^{-1} medium natural extract (MNE); 2 g.kg^{-1} high natural extract (HNE).
¶ SEM: Standard error of means
‡ D: fixed effect of dietary supplementation; T: fixed effect of time; D×T: interaction dietary supplementation x time.
[1,2,] Within a row, means without a common superscript differ ($P < 0.05$).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

CONCLUSION

In conclusion, the results of the present work underline a possible negative effect of the *Lippia* NE extract on the semen quality characteristics, besides the improvement in welfare status of the treated hares. Since a growing wide interest in dietary application of natural extract, further research is needed to assess the effect of a lower dose of *Lippia* extract on hare semen traits, taking in consideration the seminal plasma lipid peroxidation in correlation with blood oxidative markers.

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TESTICULAR ULTRASOUND AS A BREEDING SOUNDNESS EXAMINATION AND BIOMETRIC TOOL FOR WEST AFRICAN DWARF BUCK GOATS

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ABSTRACT

This study was carried out to evaluate testicular ultrasound as a breeding soundness examination (BSE) and biometric tool for West African dwarf (WAD) buck goats. Twelve bucks of proven breeding capability were selected for this purpose. Scrotal circumferences of these animals were measured using a flexible tape. Testicular ultrasound was carried out on the transverse and longitudinal planes on the right and left testes. The electronic caliper on the ultrasound machine was used to measure the testicular length, height and width from which the testicular volume was calculated using the prolate ellipsoid formula (PEF), the prolate spheroid formula (PSF) and the Lambert formula (LF). These were compared with the true testicular volumes obtained by the water displacement method (WDM). Results showed that the mean scrotal circumference was 17.48 ± 0.31 cm. Testicular echo-texture revealed homogenously greyish parenchyma with hyper-echoic mediastinum line in the middle. The scrotal wall appeared as a hyper-echoic semi-circular border line at the caudo-dorsal portion of each testis. The heads of the epididymides of the right and left testes appeared as a small roundish hypo-echoic structure on the caudo-ventral and cranio-ventral portions of each testis respectively. Results of the parameters taken using the electronic caliper revealed that the mean width of both testes was 7.24 ± 0.16 cm. There was a high correlation between scrotal circumference and width of both testes measured on the ultrasound machine. There were no statistically significant differences between the means of the left and right testes length, height and width. Similarly, differences were not statistically significant in the testicular volumes obtained using the PEF and WDM. However, the testicular volumes obtained using the PSF and LF were significantly higher than the true testicular volumes obtained using the WDM. PEF was the best of the three formulae in estimating WAD buck testicular volume. In conclusion, we suggest that testicular ultrasound will be valuable in the BSE and in measuring the important biometric parameters in WAD buck goats.

Key words: testicular ultrasound; breeding soundness examination; West African dwarf buck goat

INTRODUCTION

The West African dwarf (WAD) goats are small ruminants endowed with great breeding potential. They are good sources of animal protein in terms of meat and milk (Devendra, 1999). Nevertheless, for improved production purposes, breeding soundness examination (BSE) is a prerequisite to investigating the fertility potentials of these animals. This is particularly more important in the buck because at least five does can be bred by one buck. The ratio is even many folds higher in assisted insemination programmes (Arrebola *et al*., 2012; Ajala *et al*., 2013). BSE includes the examination for physical soundness, testicular consistency and size, semen quality and mating ability (Ridler *et al*., 2012; Menegassi *et al*., 2014). During this examination, particular attention is given to the testes as it is the site of production of sperm cells and testosterone. The sperm cells are important for the fertilization of the ova from the doe while testosterone is responsible

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for the production of sperm cells and other major WAD buck goat sexual characteristics required for efficient reproduction (Daramola *et al*., 2006; Udeh and Oghenesode, 2011). Even though in the routine BSE the testis is often palpated, the internal image or condition cannot be easily assessed. Hence, diagnosing normal and abnormal conditions of the testes is usually a difficult task. However, testicular ultrasound or sonogram offers a potential solution to this problem. It uses reflected sound waves to produce images of the testes, epididymis and scrotum. It is a safe, painless and non- invasive procedure. It does not use X-rays or other radiations and no side effects have been reported so far. The technique involves the use of a probe (transducer) to send sound waves into the testicular tissues. Reflective structures are referred to as being echogenic while the non-reflective ones are referred to as anechoic. Highly reflective structures are termed hyper-echoic while structures with low reflections are referred to as hypo-echoic (Dina Ragheb and Higgins, 2002). With this, the characteristic features of the normal and abnormal conditions of the testes and other associated structures like the epididymis can be observed. Research on testicular ultrasound has been carried out in cattle (Yimer *et al.*, 2011), camel (Pasha *et al*., 2011), sheep (Andrade *et al*., 2014), Alpine goats (Carazo *et al*., 2014) and dogs (Camara *et al*., 2014) but no such literature is available on WAD buck goats. Also, works on the use of testicular ultrasound to take into account the biometric parameters like testicular width, height and volume has not been carried out on WAD bucks. Some of these parameters are significant and have been reported to be important correlates of fertility. Testicular volume is an index of spermatogenesis. This is because ninety eight percent of the testicular volume is made up of seminiferous tubules which are responsible for spermatogenesis (Kollin *et al.*, 2006). This study was therefore carried out to evaluate testicular ultrasound as a BSE and biometric tool for WAD buck goats.

MATERIAL AND METHODS

Animals

Twelve matured healthy WAD buck goats of proven reproductive capability were selected for this study. The parameters taken and procedures carried out on these animals included:

Age: This was determined based on records from the breeders and by using the dentition formula for goats (Wosu, 2002).

Body weight: This was taken using bathroom scale (Camry®) as described by Raji and Ajala, (2015).

Scrotal circumference (SC): This was taken using a flexible tape as described earlier (Phillip and Okere, 2011; Raji and Ajala, 2015).

Testicular ultrasound and biometrics: In carrying out this, each buck was restrained by an assistant holding the animal firmly with the two limbs separated such that the testes were freely hanging caudally and at a distance of about 10 cm away from the ultrasound machine (UM). The testes were thoroughly cleaned with tissue paper before ultrasound gel (UG) was applied generously covering the entire testicular surface. The testes of the WAD buck is only covered by a thin layer of hair, hence there was no need for shaving. The UM was connected to a stabilizer which was fixed to a light source. The convex shaped transducer was then connected carefully to the monitor and switched on to the real time single B – mode at a frequency of 7.5 MHz. The UG was also applied on the probe covering the entire surface and then pressed gently on the surface of the testes. The images produced on the monitor were frozen and stored on the UM. Testicular ultrasound protocol for the bucks involved viewing the transverse plane (TP) for both testes, the right testis and the left testis; and then on the longitudinal planes (LP) for the right testis and the left testis. The testicular parameters measured were: width of both testes (W-SC) which was taken on the TP for both testes. This was measured from the most lateral point of the right testis to the most lateral point of the left testis using the electronic caliper (Figure 1). The length of the testis (L) was taken on the LP and was measured from the most cranial point on the testis to the most caudal point of the testis using the electronic caliper (Figure 2). The height of the testis (H) was measured by placing the electronic caliper from the highest ventral to the lowest dorsal points of the testes on the LP (Figure 2). The width (W) was measured as the widest diameter between the lateral and medial aspects of the testes on the TP (Figure 3). The volume of each testis was then calculated using three different formulae namely the prolate ellipsoid formula (PEF) – $L \times H \times W \times 0.52$ cm^3, the prolate spheroid formula (PSF) – $L \times W^2 \times 0.52$ cm^3 and the Lambert formula (LF) – $L \times H \times W \times 0.71$ cm^3. These were compared with the true testicular volumes using the water displacement method as described by Mbaeri *et al*. (2012). The parameters measured were recorded and analyzed (Sotos and Tokar, 2012). The UM used for this study was Biocare Ultrasonic Diagnostic Equipment (Model: BU – 907). Images were displayed on grey scale.

Statistical analysis

The data obtained in this study were analyzed using the Pearson Product Moment Correlation (PPMC) and the Student t-test at the level of significance $P < 0.05$ using SPSS version 20.

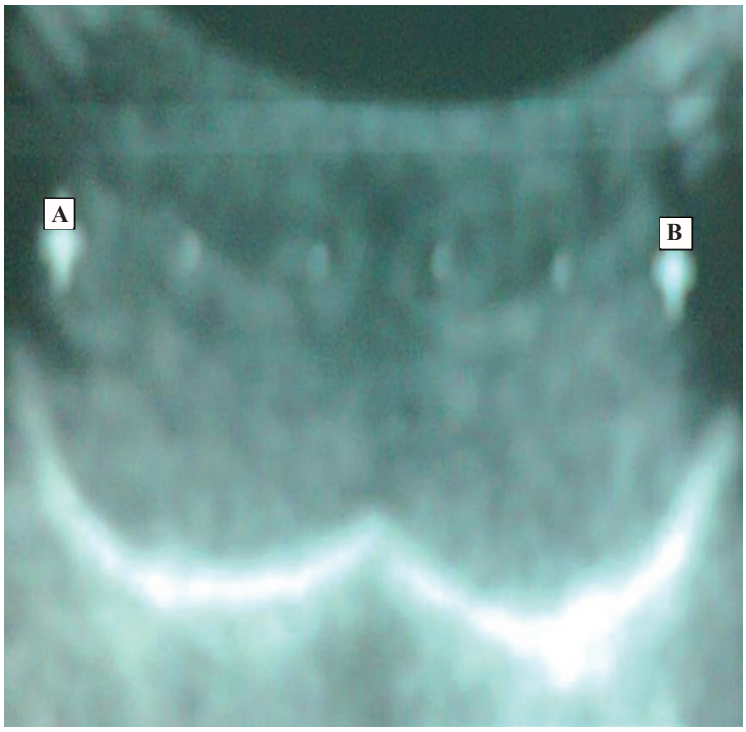

Fig. 1: Sonogram showing the width of both testes (A - B) on the transverse plane

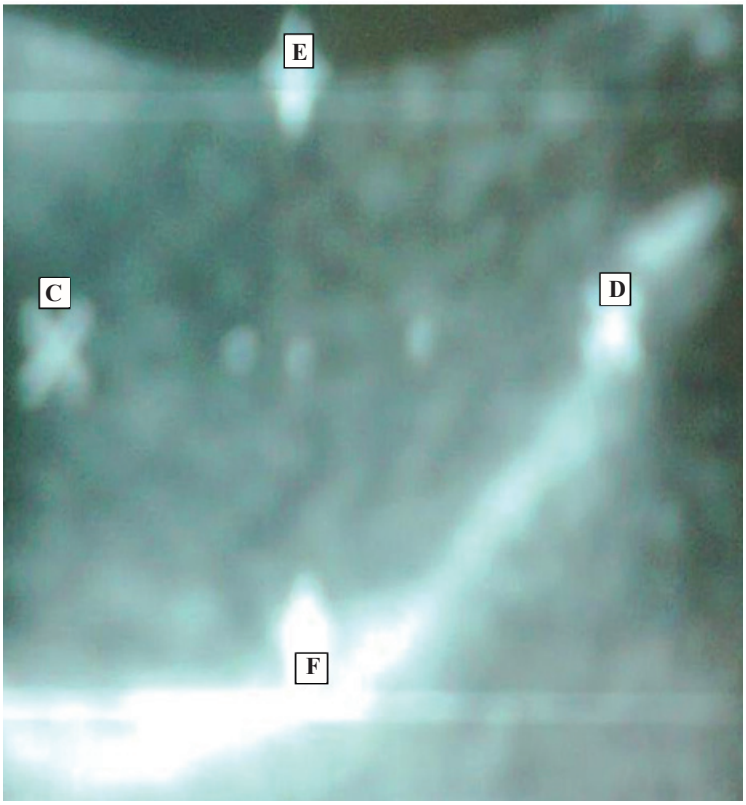

Fig. 2: Sonogram showing the length (C-D) and height (E-F) of the right testes on the longitudinal plane

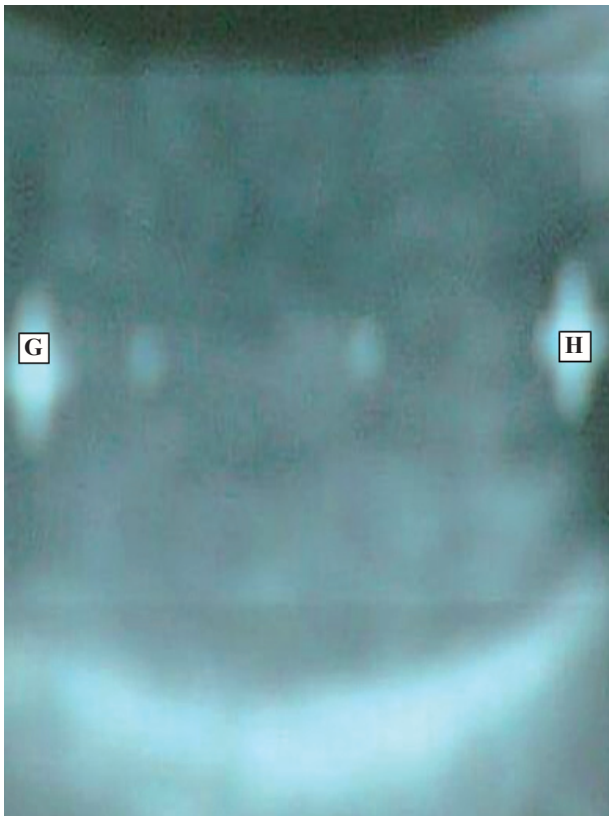

Fig. 3: Sonogram showing the width of testis (G-H) on the transverse plane

RESULTS

The bucks studied were between the ages 1 and 2 years. The average body weight and scrotal circumference observed were 12.6 ± 0.57 kg and 17.48 ± 0.31 cm respectively. Testicular ultrasound revealed that on the TP and LP for the right and left testes, testicular parenchyma appeared homogenously greyish while the mediastinum testes appeared as a white hyper-echoic thin line in the mid-section of the testes. The scrotal wall appeared as a thick hyper-echoic semi-circular layer forming a border line at the caudo-dorsal portion of each testis on the TP (Figure 4). The head of the epididymis of the right testis appeared as a small roundish dark hypo-echoic structure on the caudo-ventral part of the testes on the LP (Figure 5); while the head of the epididymis for the left testis appeared similar to that of the right testis but on the cranio-ventral portion of the testes also on the LP (Figure 6). The results for width of both the testes (W-SC), length, height and width of the right and left testes are as presented in Table 1. The mean W-SC was 7.24 ± 0.16 cm. This was highly correlated to the SC – 17.48 ± 0.31 cm ($r = 0.94$; $p \leq 0.01$). There were no significant differences between the means

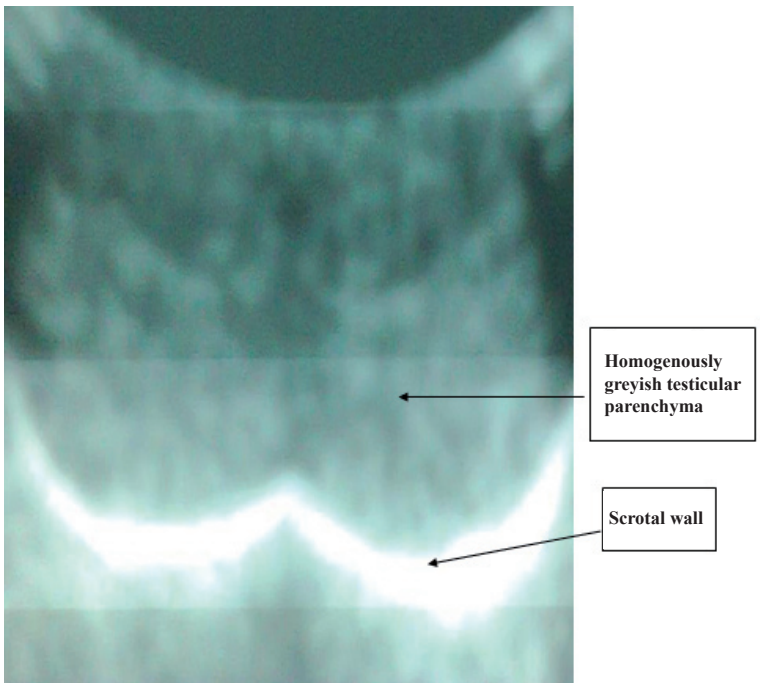

Fig. 4: Sonogram showing the homogenously greyish testicular parenchyma, and scrotal walls on the transverse plane of the right and left testes of the control group A buck

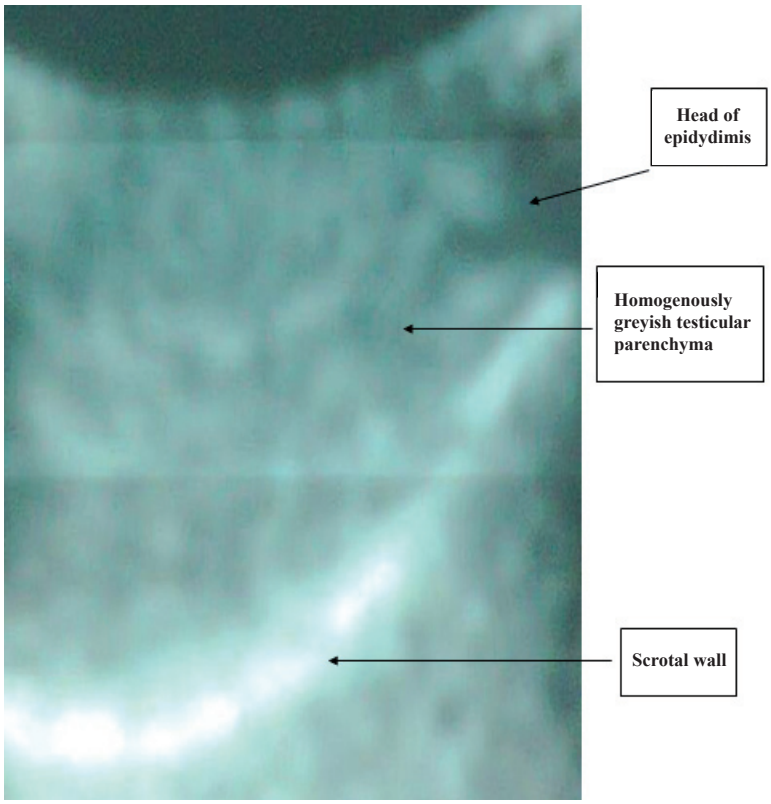

Fig. 5: Sonogram showing the head of the epidydimis, testicular parenchma and scrotal wall of the right testis on the longitudinal plane

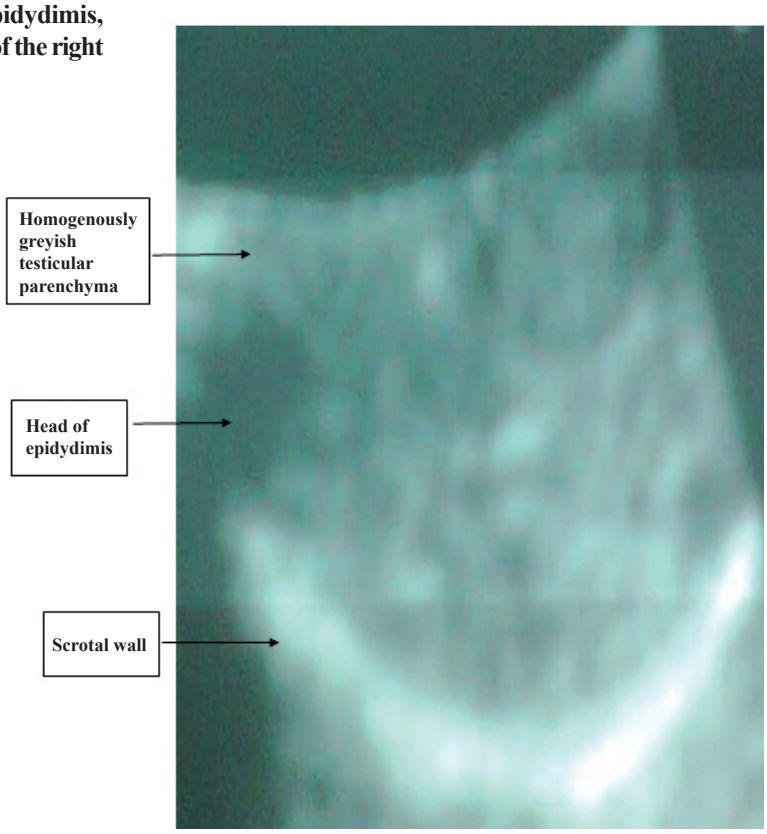

Fig. 6: Sonogram showing the head of the epidydimis, testicular parenchyma and scrotal wall of the left testis on the longitudinal plane

of the left and right testes length, height and width ($p = 0.79$, $p = 0.81$, $p = 0.82$, at the level of significance $p < 0.05$) respectively. The result for the testicular volume for the WAD buck goats are as presented in Table 2. There were no significant differences between the means of the true testicular volumes obtained by the WDM and the testicular volume obtained using the PEF for the right testis ($p = 0.83$) and left testis ($p = 0.96$) respectively. Similarly, no significant differences could be noted when the testicular volumes obtained by PSF were compared with those obtained using the LF for the right testis ($p = 0.65$) and the left testis ($p = 0.47$), respectively. However, the testicular volumes calculated by using the PSF were significantly higher than the true testicular volumes obtained by the WDM for the right testis ($p = 0.02$) and the left testis ($p = 0.01$), respectively. Similarly, no significant differences were observed when the testicular volumes obtained using the LF were compared with those obtained by the WDM for the right testis ($p = 0.03$) and the left testis ($p = 0.02$), respectively. The testicular volumes calculated by using the PSF were significantly higher than the testicular volumes obtained by the PEF for the right testis ($p = 0.02$) and the left testis ($p = 0.01$), respectively. Similarly, there were no significant differences when the testicular volumes obtained using the LF were compared with those obtained by the PEF for the right testis ($p = 0.02$) and the left testis ($p = 0.02$), respectively.

DISCUSSION

This study was conducted to evaluate testicular ultrasound as a BSE and biometric tool for WAD buck goats. The age, weight and scrotal circumference (SC) of the WAD buck studied were similar to those reported in matured WAD buck goats by Ugwu (2009). We also observed that the testicular echo-texture of the WAD buck goats were similar to those reported in fertile bulls (Ali *et al.*, 2011), rams (Ulker *et al*., 2005), camels (Pasha *et al*., 2011) and Alapine goats (Carazo *et al*., 2014).

The width of both the testes (W-SC) measured on the ultrasound machine was highly correlated to the SC measured with the flexible tape. We suggest that this could be taken as the SC when performing testicular ultrasound. Further studies should be carried out in this species and other species and breeds of animals to establish and further standardize this finding. We suggest this as a new finding to be adopted in the improved clinical approach to BSE of WAD buck goats.

Table 1: The testicular width of both testes, length, height and width of the of the right and left testis of West African Dwarf bucks measured using the electronic caliper on ultrasound machine

Goats	LRT (cm)	HRT (cm)	WRT (cm)	LLT (cm)	HLT (cm)	WLT (cm)	WDBT (cm)
1	6.03	2.61	3.65	5.91	2.52	3.49	7.14
2	5.98	2.53	3.50	6.03	2.54	3.70	7.20
3	5.87	2.58	3.44	5.89	2.58	3.66	7.10
4	6.19	2.57	3.73	6.07	2.56	3.48	7.21
5	5.92	2.48	3.57	5.88	2.51	3.81	7.38
6	5.83	2.54	3.71	5.79	2.52	3.53	7.24
7	6.23	2.59	3.65	6.12	2.53	3.69	7.34
8	5.97	2.50	3.40	5.91	2.49	3.54	6.94
9	5.83	2.61	3.76	5.80	2.59	3.80	7.56
10	6.16	2.50	3.59	6.07	2.50	3.62	7.21
11	5.94	2.55	3.61	5.86	2.56	3.60	7.21
12	5.87	2.56	3.72	5.92	2.51	3.64	7.36
	5.99	2.55	3.61	5.94	2.53	3.63	7.24
	± 0.14	± 0.04	± 0.12	± 0.11	± 0.03	± 0.11	± 0.16

$P < 0.05$ level of significance.
LRT- length of right testis, HRT – Height of right testis, WRT – width of right testis, LLT – length of left testis, HLT – height of left testis, WLT – width of left testis, WDBT – width of both testis.

Table 2: The testicular volume of West African Dwarf bucks taken using the water displacement method, prolate ellipsoid formula, prolate spheroid formula and Lambert formula (mean ± standard deviation)

Goats	Right Testis				Left Testis			
	VWDM (cm^3)	VPEF (cm^3)	VPSF (cm^3)	VLF (cm^3)	VWDM (cm^3)	VPEF (cm^3)	VPSF (cm^3)	VLF (cm^3)
1	28.00	29.87	41.77	40.79	29.00	27.03	37.43	36.90
2	29.40	27.54	38.09	37.60	27.90	29.47	42.93	40.24
3	28.80	27.09	36.12	36.99	28.30	28.92	41.03	39.49
4	27.90	30.86	44.78	42.13	28.00	28.12	38.23	38.39
5	29.10	27.25	39.23	37.21	28.70	29.24	44.38	39.92
6	28.90	28.57	41.73	39.01	28.40	26.78	37.52	36.59
7	28.60	30.63	43.16	41.82	27.90	29.71	43.33	40.57
8	28.00	26.39	35.89	36.03	28.40	27.09	38.51	36.99
9	28.50	29.75	42.86	40.62	27.90	29.68	43.55	40.53
10	29.30	28.75	41.28	39.25	28.40	28.57	41.36	39.00
11	27.90	28.43	40.25	38.82	28.10	28.08	39.49	38.34
12	28.60	29.07	42.24	39.69	29.40	28.13	40.79	38.40
	28.58^{*acd}	28.68^{*bcd}	40.62^{*cab}	39.16^{*dab}	28.37^{*egh}	28.40^{*fgh}	40.71^{*gef}	38.78^{*hef}
	± 0.54	± 1.43	± 2.79	± 1.95	± 0.47	± 1.04	± 2.47	± 1.42

P < 0.05 level of significance.. *Significant acd i.e when column a is compared with column c and d respectively etc.
VWDM – volume by water displacement method, VPEF – volume by prolate ellipsoid formula,
VPSF – volume by prolate spheroid formula, VLF – volume by Lambert formula.

The testicular ultrasound biometric study also revealed that the mean length, height and width of the right testis were 5.99 ± 0.14 cm, 2.53 ± 0.04 cm and 3.61 ± 0.12 cm, respectively; and the left testis were 5.94 ± 0.11 cm, 2.53 ± 0.03 cm and 3.63 ± 0.11 cm, respectively. There were no significant differences when these parameters were compared between the right and the left testis. These are similar to the reports in humans but with some variation in the values of these parameters, probably due to species variation (Behre *et al*., 1989; Kiridi *et al*., 2012). However, reports on these testicular biometric parameters are scarce in the animal species. Further studies should be carried out for comparison and most importantly for improved production. This is the first report on these testicular ultrasound biometric parameters in WAD buck goat as far as the literature suggests. Also, the mean testicular volumes by VWDM, PEF, PSF and LF for the right testis were 28.58 ± 0.54 cm^3, 28.68 ± 1.43 cm^3, 40. 62 ± 2.79 cm^3 and 39.16 cm^3, respectively; and those for the left testis were 28.37 ± 0.47 cm^3, 28.40 ± 1.04 cm^3, 40.71 ± 2.47 cm^3 and 38.78 ± 1.42 cm^3, respectively. The PEF estimated testicular volumes closest to the true testicular volumes obtained by WDM without any significant difference. However, the PSF and LF over-estimated the testicular volume in these WAD buck goats, in our own view. We suggest that the PEF is the best of the three formulae, in estimating the testicular volume of the WAD buck breed of goat. Further studies should be carried out to fully establish this in these animals and other breeds and species as well. Souza *et al*. (2012) used the PEF to estimate testicular volumes in canine species. But they did not compare this with the other formulae and methods used by us in this study. However, Paltiel *et al.* (2002) compared these three formulae also in canine species and recommended the LF as the preferred formula for clinical practice. In humans, the LF was also reported to be the preferred formula for estimating testicular volume (Sotos and Tokar, 2012; Kiridi *et al*., 2007). This variation may be due to species differences but as far as this study is concerned, as suggested, the PEF is the preferred formula for testicular volume estimation in the WAD buck goat. These findings for the first time suggest these as valuable source of animal protein thus offering a new, more accurate, none invasive method of evaluating testicular volume which can be adopted to replace the invasive methods which require the removal of testes before testicular volume can be calculated (Franca *et al*., 2000). These alternatives offered by the use of TU in evaluating important testicular

parameters such as SC and TV will lead to a better clinical approach to predicting fertility potentials and possible rapid diagnosis of infertility problems that are related to testicular dysfunctions in WAD buck goats. These are accrued to the fact that SC has been reported to be a significant correlate of fertility in buck goats (Bongso *et al*., 1982; Raji *et al*., 2008; Ugwu, 2009). Also, TV has been documented to be an important index of spermatogenesis because about 98 % of the testis is made up of the seminiferous tubules where sperm cells are produced (Kollin *et al*., 2006).

CONCLUSION

In conclusion, this study has shown that testicular ultrasound is a potentially valuable tool in the BSE and in measuring important testicular biometric parameters such as SC and testicular volume particularly by using the prolate ellipsoid formula (PEF) in WAD buck goats. Therefore, we suggest that its use and introduction into the BSE programmes of these bucks should be encouraged. Adoption and use of the findings of this study can go a long way in improving the WAD buck goat production thereby leading to availability of more supply of animal proteins.

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NUTRIENT CONTENT AND ORGANIC MATTER DEGRADABILITY OF DIFFERENT MORPHOLOGICAL PARTS OF MAIZE HYBRIDS DENT AND DENT X FLINT

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ABSTRACT

The aim of our study was to determine the nutrient content and organic matter degradability of different morphological parts (whole plants, stalks, leaves) of maize hybrids dent and dent x flint. Ruminal degradability was determined by *in sacco* method. Hybrids dent x flint - Mesnil, Chambord, Queen, and hybrids dent - Aude, Meridien, KX 1393, Omero were used.
The content of ADF, NDF and lignin in the stalks was higher in the dent than in dent x flint hybrids. Concentration of crude protein (CP) in leaves was two times higher than in stalks (117.0 $g.kg^{-1}$ DM and 53.0 $g.kg^{-1}$ DM on average, respectively). The differences were also noted in CP among the hybrids in all plant parts. Large differences were found in starch content among the hybrids in whole plants: in Mesnil it was 329.0 $g.kg^{-1}$ of DM, whereas in Meridien the starch content was 193.0 $g.kg^{-1}$ of DM only.
In sacco experiment was carried out on three rumen cannulated cows. Hybrids dent x flint had on average higher effective organic matter degradability in whole plants (56.1 %), stalks (38.8 %) and leaves (49.2 %) than hybrids dent (53.8 %, 35.2 % and 43.3 %). Also, the rate of degradation of organic matter (OM) was higher for hybrids dent x flint than for dent. Organic matter in the stalks was degraded more slowly than in leaves.

Key words: morphological parts; maize plant; dent; dent x flint; organic matter; rumen degradability; *in sacco* method

INTRODUCTION

Maize is an important carbohydrate feedstuff by virtue of its rich chemical composition and nutrient content. Maize is characterized by high content of energy, which is a basic assumption of nutrition, although it does not cause abnormalities, but significantly reduces the utility (Sommer *et al*., 1985).

Individual morphological parts of the plant maize, according Struik (1983), are as follows: 43 % grain, 16 % leaves, 1 % panicle, 10 % stems and 12 % bracts, and contain several other nutrients which implies the various contents of energy. The vegetative components (stalk, leaf, husk and cob) can constitute approximately 70 % of the whole plant dry matter and can affect the quality of forage from the maize plant (Caetano *et al*., 2011).

Some studies have shown that in addition to the grain, the vegetative components of the maize plant are also important in the improvement of forage quality (Silva *et al.*, 2008). The concentration of crude protein, fat, non-fibre carbohydrate, neutral detergent fibre and the digestibility of these nutrients influence the energy value of feedstuffs (Weiss, 1994).

In the assessment of the feed quality for ruminants it is important to examine the degradability of nutrients in the rumen. Effective degradability characterizes the changes of feed, the kinetics of its degradation, taking into account the rate of passage from the rumen to duodenum (Ørskov and McDonald, 1979). *In sacco* method allows to obtain these data for several feeds at the same time.

The aim of our study was to determine the nutrient

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content of different morphological parts of maize hybrids dent and dent x flint and degradability of organic matter in different morphological parts of maize by *in sacco* method.

MATERIAL AND METHODS

Maize hybrids with the type dent (Aude, Meridien, KX 1393, Omero) and dent x flint (Mesnil, Chambord, Queen) were used in our experiment. Oganic matter degradability in the morphological parts of maize was determined by *in sacco* method (Harazim and Pavelek, 1999). All the maize hybrids are stay green with different FAO (Table 1).

The samples of maize hybrids were harvested at the time of milk-waxy maturity. The samples were divided into different parts whole plants (stalks + leaves + stems), leaves and stalks. In the whole plant and individual morphological parts original dry mater (DM) and chemical composition were determined. Materials designed to degradability determination were freeze-dried and ground. These samples were weighed (approx. 2.50 g dry matter) into bags (9 x 15 cm) made of Uhelon 130T (HEDVA, "Moravská Třebová", the Czech Republic) with pore size of 47 μm. Minimum of three separate bags for hybrids, incubation time and animals were used. The bags with samples were incubated for 2, 3, 4, 6, 9, 16, 24, 48, 72 and 96 hours. The 0 h time bags were only washed in water to determine washing losses.

In sacco experiments were carried out in three non-lactating cows with large rumen cannulae (an average of 10 cm). The animals were fed twice a day with a diet consisting of 70 % forage and 30 % concentrate on a dry matter basis at maintenance level. Nutrient intake to one cow/day in our experiment was followed: 9770 g dry matter; 1170 g crude protein; 5050 g nitrogen free extract; 2660 g fibre; 1980 g starch and 650 g ash. Access to water was *ad libitum*.

Fifteen mg samples were inserted into the rumen just before morning feeding. The content of organic matter was determined in morphological parts of maize and in the residues after all incubation times. The content of nutrients was analyzed according to AOAC (2000). Contents of ADF, NDF and lignin were determined according to Van Soest (Lutonská and Pichl, 1983). The parameters of degradability (a, b, c, and "effective degradability") were calculated using the equations by Ørskov and McDonald (1979) with outflow rate of $0.06.h^{-1}$.

The data on nutrient content and organic matter (OM) degradability were evaluated statistically (mean and standard deviation). The statistical package Statistix 8.0 was used for statistical methods. Statistical evaluation of the results was performed by the one-way ANOVA and Tukey test for multiple comparisons at the level of significance $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

The nutritional value of different morphological parts of the plant is decreased with increasing maturity (Pesche and Gross, 1980). It reduces the nutritional value of whole maize plant. At the time of maize harvesting (milk – waxy stage), leaves had higher content of dry matter than stalks, regardless of the type of hybrids. Small differences were found only for dry matter of whole plants except for hybrid Queen and Aude (Table 2).

Differences among hybrids in the nutrient content of whole plants and dry matter are not caused only by actual differences between morphological parts, but also shared by various morphological parts of ripeness stage at harvest (Verbič *et al*., 1995). Harika *et al*. (1995) asserted that the quality of maize stover depends on the proportions of leaf and stem fractions of the stover.

Table 1: Characteristics of maize hybrids

Hybrid	FAO	Type of corn	Type of hybrid
Mesnil	290/300	dent x flint	Sc
Chambord	300/300	dent x flint	Sc
Queen	320/340	dent x flint	Sc
Aude	380/380	dent	Sc
KX 1393	450/450	dent	Sc
Meridien	420/420	dent	Sc
Omero	480/480	dent	Sc

Table 2: Nutrient content of morphological parts of selected maize hybrids (g.kg^{-1} DM)

	Dry matter			Crude protein			Organic matter			Starch	ADF			NDF			Lignin		
Maize hybrids	WP	S	L	WP	S	L	WP	S	L	WP	WP	S	L	WP	S	L	WP	S	L
Mesnil	374	228	268	78	54	144	965	959	911	329	232	363	287	429	585	537	23	45	25
Chambord	372	281	323	74	35	116	964	965	855	246	231	380	282	436	599	520	28	46	26
Queen	403	287	356	83	52	108	957	947	896	312	252	462	294	455	737	559	32	49	21
Aude	436	300	384	80	57	101	953	952	883	261	246	382	296	480	646	562	27	32	25
KX 1393	375	275	338	90	52	126	953	960	898	205	258	414	314	490	683	576	29	46	29
Meridien	375	274	337	84	62	122	955	951	903	193	287	455	318	566	714	589	24	55	23
Omero	376	234	370	87	56	104	948	938	882	253	280	440	336	549	721	603	30	51	37

WP – whole plants, S – stalks, L – leaves, ADF – acid detergent fibre, NDF – neutral detergent fibre

Table 3: Statistical comparison of nutrient content in maize hybrids at the level of significance $P < 0.05^{*}$ and $P < 0.01^{**}$

Nutrients	Whole plants	Stalks	Leaves
ADF	1:(3,4,5,6,7)**, 2:(3,4,5,6,7)**, 3:(7,6)** 4:(5,6,7)**, 5:(6,7)**, 6:7*	1:(3,4,5,6,7)**, 2:(3,5,6,7)** 3:(4,5,7)**, 4:(5,6,7)** 5:(6,7)**, 2:1*, 7:6*	1:(5,6,7)**, 2:(5,6,7)**, 3:(5,6,7)**, 4:(5,6,7)**, 7:(5,6)** , 2:(3,4)*
NDF	1:(3,4,5,6,7)**, 2:(4,5,6,7)**, 3:(4,5,6,7)** 4:(6,7)**, 5:(6,7)**, 2:3*	1:(3,4,5,6,7)**, 2:(3,4,5,6,7)** 3:(4,5,6)**, 4:(5,6,7)** 5:(6,7)**, 3:7*, 1:2*	1:(3,4,5,6,7)**, 2:(3,4,5,6,7)**, 3:(6,7)**, 4:(6,7)**, 5:7**, 1:2*, 3:5*
Lignin	3:(1,6)**, 7:1**, 1:(2,5)*	4:(1,2,3,5,6,7)**, 6:(1,2,3,5)** 7:1**, 7:(2,5)*	1:(3,7)**, 2:7**, 3:(5,7)**, 7:(4,5,6)**, 6:(1,5)*, 3:2*
Crude protein	1:(5,6,7)**, 2:(3,4,5,6,7)**, 3:5**, 4:(5,7)**, 5:6** 1:(2,3)*, 3:7*, 4:6*	1:(2,6)**, 2:(3,4,5,6,7)** 6:(3,5)**, 4:(3,5)*, 7:(5,6)*	1:(2,3,4,5,6,7)**, 2:(3,4,5,7)**, 3:(5,6)**, 4:(5,6)**, 7:(5,6)**, 2:6*, 3:4*

1 – Mesnil, 2 – Chambord, 3 – Queen, 4 – Aude, 5 – KX 1393, 6 – Meridien, 7 – Omero

Table 4: Characteristics of degradability and effective degradability of organic matter in morphological parts of selected maize hybrids

Parameter		Maize hybrids						
		Mesnil	Chambord	Queen	Aude	Meridien	KX 1393	Omero
a (%)	WP	41.3^{d}	37.8	28.3^{bd}	34.6^{a}	46.9^{abc}	37.1	35.1^{c}
	S	27.7	31.0	27.5	25.8	25.2	25.6	23.7
	L	36.3	32.3	23.2	34.7	25.6	27.0	29.6
b (%)	WP	41.0	43.7	52.7	45.8	42.0	48.9	49.6
	S	32.2	36.2	46.1	37.5	44.5	37.8	43.8
	L	49.6	51.7	55.2	52.4	55.5	59.1	48.6
c (%.h^{-1})	WP	0.056^{c}	0.042	0.063^{ab}	0.047	0.021^{c}	0.027^{a}	0.036
	S	0.046	0.027	0.024	0.022	0.023	0.037	0.028
	L	0.031	0.038	0.070	0.021	0.028	0.030	0.038
Edg (%)	WP	56.4^{d}	56.1^{c}	55.9^{b}	55.3^{a}	54.6	51.9^{abcd}	53.2
	S	40.4^{abcdef}	40.1^{eghijk}	36.0^{bh}	33.9^{ag}	35.1^{ej}	36.8^{di}	35.0^{fk}
	L	52.8	49.0	49.1	41.9	43.2	42.9	45.2

WP – whole plants, S – stalks, L – leaves, Edg – effective degradability. Means with the same letters in the same row are significantly different at $P < 0.05$ and $P < 0.01$.

Starch in whole plants was the highest in hybrid Mesnil (329 g.kg^{-1} DM) and the lowest in Meridien (193 g.kg^{-1} DM).

The differences in the contents of ADF, NDF and lignin were found by Kohler *et al*. (1990) among the hybrids as well as between morphological parts. It corresponds with our results (Table 2). Higher contents of ADF, NDF and lignin were found in the stalks of dent hybrids than dent x flint hybrids (Table 2), except hybrid Queen. The similar tendency was observed also in leaves and whole plants. Tolera and Sudstøl (1999) noted the highest contents of crude fibre, ADF, NDF and lignin in stalks.

The concentration of crude protein in leaves ranged from 101 to 126 g.kg^{-1} DM of hybrids dent and from 108 g.kg^{-1} to 144 g.kg^{-1} DM of dent x flint hybrids. The results indicate that the maize leaves have about three times more crude protein than stalks. Average crude protein content in whole plant was higher in dent hybrids as in dent x flint hybrids (85 g.kg^{-1} vs. 78 g.kg^{-1} DM). However, the quality of maize proteins is poor because they are deficient at the essential amino acids lysine and tryptophan (Shewry, 2007). Significant differences between hybrids in content of nutrients are presented in Table 3.

Among the morphological parts of maize plants and also among maize hybrids there are differences in the chemical composition, which results in the differences of the effective organic matter degradability. Many authors (Liu *et al*. 1988, Negi *et al*. 1988; Susmel *et al*. 1990; Mir *et al*. 1991) referred to the differences in degradability of morphological parts of maize plant. According to Verbič *et al*. (1995), it can be used in the selection of suitable hybrids for ensilaging.

The effective OM degradability (Edg) was found to be the highest for whole plants of maize (from 51.9 to 56.1 %) (Table 4). The differences were statistically significant between the hybrids dent x flint and hybrid dent KX 1393 (Table 4). The effective OM degradability for leaves was in the range from 41.9 to 52.8 % but they were not statistically significant. A higher amount of lignin in the stalks was reflected in low levels of all parameters of OM degradability. Particularly in the fraction "a", effective OM degradability was lower in the stalks than in leaves and whole plants. The differences among dent and dent x flint hybrids were significant for parameters "a" and "c", the effective OM degradability in the whole plants and for Edg (effective degradability) in the stalks.

The rate of degradation "c" of the insoluble fraction "b" was the highest in the leaves (Table 4). The higher content of lignin reduces the degradation of cell walls in the rumen, but does not affect the loss of soluble substances such as sugar. We found that the hybrid Meridien with the highest concentration of

lignin in whole plants and stalks had the lowest rate of degradation (parameter c) of organic matter. Higher degradability of leaves compared with stems was reported for most cereals (Kernan *et al*., 1984; Ramanzin *et al*., 1986; Shand *et al*., 1988).

CONCLUSION

The content of nutrients was different in hybrids and changed with morphological parts of maize hybrids. We found the lowest effective degradability of organic matter in stalks followed by the leaves and the highest effective degradability of OM was in the whole plants. From our results it may be concluded that there are differences in chemical composition and differences in effective degradability of maize between morphological parts of the maize plant as well as among maize hybrids. Therefore it is necessary to select the correct maize hybrid on the basis of objectively determined nutritive value.

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EFFECT OF DIETARY ZINC SUPPLEMENTATION ON NUTRIENTS DIGESTIBILITY AND FERMENTATION CHARACTERISTICS OF CAECAL CONTENT IN PHYSIOLOGICAL EXPERIMENT WITH YOUNG RABBITS

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ABSTRACT

The effects of orally administered zinc from inorganic or organic sources on selected parameters of nutrient digestibility and caecal fermentation pattern in rabbits were the priority of this study. A total of 96 weaned rabbits (35th day of age, both male and female) were divided into 4 groups (control C and three experimental groups – 1EG, 2EG and 3EG) with 24 animals in each group. Maternal albinotic line (crossbreed New Zealand White, Buskat Rabbit, French Silver) and paternal acromelanic line (crossbreed Nitra Rabbit, Californian Rabbit, Big Light Silver) were used. The feed mixture was additionally administered as follows: in the 1st experimental group 1EG by a dose of 27.47 g $ZnSO_4.H_2O$ (zinc sulphate monohydrate), in the 2nd group (2EG) by a dose of 38.46 g Glycinoplex-Zn and in the 3rd group (3EG) a dose of 66.67 g Bioplex-Zn, each per 100 kg. Rabbits were fed with complete pelleted mixtures *ad libitum* and had free access to water via a nipple drinker. Dietary supplementation of rabbits with zinc was carried out to determine its effects on growth of live weight and consumption of feed per unit of live weight growth. Between 77-81 days of age, four rabbits from each group were selected for digestibility tests using the balance method. On the 91st day of age (6 weeks after all experimental procedures), 6 animals from each group were slaughtered, caecum and appendix were separated, and the caecal samples were collected for analysis; pH, VFA, ammonia-N and lactic acid were determined. We did not find any differences among experimental groups in the digestibility coefficients of starch, N-Free Extract, organic matter and Ca, P, Mg, and Cu obtained through the balance method ($P > 0.05$), compared with the control group or those fed with 100 Zn mg.kg^{-1} supplemented diets. Increase in the supplemental Zn level to 100 mg.kg^{-1} diet resulted in significant increase in digestibility coefficients of Na, K, Fe, Mn ($P < 0.05$) and Zn ($P < 0.01$) compared to the control group. No significant effect of the diet was detected on caecum in relative weights of its content, as well as on dry matter content. Feeding of rabbits with inorganic or organic zinc sources did not influence selected biochemical parameters in caecal fermentation, as well as had no negative effect on the rabbit growth performance.

Key words: rabbits; zinc; digestibility of nutrients; caecal fermentation pattern

INTRODUCTION

Zinc (Zn) has an important role in numerous biological processes. Zinc is an essential component of many enzymes (for the activity of over 300 enzymes), and it has both structural and catalytic functions in metalloenzymes (McCall *et al.*, 2000). One of the most important functions of Zn is related to its antioxidant role and its participation in the antioxidant defence system. The mechanism, by which Zn exerts its antioxidant action, is not well defined. However, it has been suggested that Zn increases the synthesis of metallothionein, a cystine-rich protein that acts as

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a free radical scavenger (Oteiza *et al*., 1996).

Zinc is absorbed in the small intestine and an intestinal pool of Zn may be formed by binding the metal to the intestinal metallothionein or Zn may be transported by albumin plasma to the liver (Prasad, 1993). More than one isoform of metallothionein is found in different tissues of animal species. Recently, a family of Zn transporters that play an important role in the regulation of Zn metabolism at the intracellular level in mammalians has been described. They structurally consist of 6 transmembrane domains, an intracellular histidine-rich region, and the amino and carboxy terminus, which resides intracellularly (Tako *et al*., 2005).

On the other hand, a single isoform of metallothionein in the chicken has been found in liver, pancreas, kidney and intestinal mucosa (McCormick, 1984; Sandoval *et al*., 1998). Metallothionein is synthesized in tissues in response to dietary Zn and can bind 7 atoms of Zn per molecule of protein, but they can also bind Cu with a higher affinity (Cousins and Lee-Ambrose, 1992).

Several biochemical and different clinical manifestations of Zn deficiency have been reported. Blood Zn concentrations are lower and the activity of several enzymes in metabolic pathways decreases in Zn-deficient animals. Zinc deficiency causes a loss of appetite and reduced efficiency of feed utilization and thus leads to growth retardation (Ensminger *et al*., 1990; Mc Dowell, 2003).

Zinc deficiency in animals is characterized by decreased feed intake, decreased growth, low circulating levels of growth hormone (GH) and insulin-like growth factor-I, and decreased hepatic production of insulin-like growth factor-I, GH receptor and GH binding protein. Zinc positively affects feed utilization through participating in the metabolism of carbohydrates, lipids, and proteins (McDonald, 2000). Minerals activate enzymes and they are essential cofactors of metabolic reactions, and function as carriers of proteins, regulate digestion, respiration, water balance, muscle response, the neural transmissions, influence and maintain skeletal strength, balance pH, and even mental balance, protect against disease, act as antagonists or synergists of other elements and play a vital role in the resistance, adaptation and evolution of new races and lines (Anke *et al*., 1988; Szentmihalyi *et al*.,1985; Haenlein, 1987).

Because of many natural food ingredients show marginal Zn-deficiency, this micronutrient is commonly supplemented to diets for livestock and poultry. Regardless of the fact that certain microelements are present in sufficient quantities in food, subclinical or clinical symptoms of their deficiency appear. This can be cause of their different and changeable availability, or the microelements are present in form that cannot be used. Obtained results showed that the presence of certain substances in food (phytic acid and oxalic acid), as well as interaction with other nutrients in the digestive tract, influencing resorption mechanisms. Resorption of microelements is not dependent only on their content in food, but also on the animals' age, electrochemical reactions in the intestine, and on the microelement form. Mineral salts are most frequently used, such as oxides, carbonates, chlorides and sulphates. Today, in addition to inorganic forms of minerals, the use of so-called „chelate" forms, i.e. organically bound microelements, is becoming more frequent.

The effects of orally administered zinc from inorganic or organic sources on selected parameters of nutrient digestibility and caecal fermentation pattern in rabbits were the priority of this study.

MATERIAL AND METHODS

Animals

A total of 96 rabbits (35th day of age, both sexes) were randomly divided into four groups (control C and three experimental groups – EG) with 24 animals in each group. The rabbits of meat line M91, maternal albinotic line (crossbreed New Zealand white, Buskat rabbit, French silver) and line P91, paternal acromelanic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in this experiment. Rabbits were housed in standard cages (0.61 m x 0.34 m x 0.33 m) with two animals per cage. The cages allowed the separation of faeces. The environmental temperature ranged from 24 °C to 31 °C, relative humidity of 65 %; these values were recorded continually using thermograph positioned at the same level as the cages. A cycle of 16 hours light and 8 hours of dark was used throughout the experiment. The animals were healthy and their condition was judged as good at the beginning of the experiment. In this animal study institutional and national guidelines for the care and use of animals were followed. Each experimental procedure, which involves animals, was approved by the State Veterinary and Food Institute of the Slovak Republic.

Experimental design

The animals were fed with complete pelleted feed (pellets of 3.5 mm in diameter) ad libitum and had free access to water via a nipple drinker. The diets were composed of 36 % dehydrated lucerne meal, 5.5 % extracted sunflower meal, 5.5 % extracted rapeseed meal, 9 % wheat bran, oats 13 %, malt sprouts 15 %, DDGS (dried distillers grains with soluble) 5 %, sodium chloride, mineral and vitamin mixture*, barley grains 8 %, limestone 1 %. The diet did not contain any anti-coccidial drug. The feed mixture was additionally administered:

in the 1st experimental group by dose of 27.47 g $ZnSO_4 . H_2O$ (Zinc sulphate monohydrate), in the 2nd group (2EG) by dose of 38.46 g of Glycinoplex-Zn and in the 3rd group (3 EG) dose of 66.67 g Bioplex Zinc, each per 100 kg. The rabbits in the group C were fed with the same commercially available diet with no zinc additive. The chemical composition of all feeds was determined by Weende (AOAC, 2000). The fattening experiment lasted for 48 days.

Dietary supplementation of rabbits with zinc was carried out to determine its effect on live weight growth and consumption of feed per unit of live weight growth. Rabbit's body weight and feed consumption were measured every week of the experiment. Mortality and morbidity were also recorded in the groups daily, over the entire period of the experiment. In the morning on 91st day of age (6 weeks after all experimental procedures) 6 animals from each group were electrically stunned and killed by cutting the carotids and jugular veins, then the carcasses were refrigerated for 24 h at 4 °C. Raw meat samples were packed and stored at -25 °C until they were analyzed. Caecum and appendix were separated and sampled for biochemical analyses.

Digestibility study

Total tract apparent digestibility was measured according to E.G.R.A.N. (2001), four rabbits (males, 2550 ± 100 g live body of weight) from each group were housed individually in metabolic cages (between 77 - 81 days of age). The adaptation period for this diet was 28 days. The faeces were collected individually during four consecutive days according to the European reference method for rabbit digestion trials (Perez *et al*., 1995). Sampling of faeces was performed every 2 hours. Faeces were collected in bags during the day time. Every day, in the morning, faeces were mixed with a handy mixer, the average samples were pre-dried (at 60 °C for 36 h in a dryer) and grinded (1 mm screen) with laboratory grinder for chemical analysis. Chemical analyses were conducted according to AOAC (2000) with the considerations mentioned by E.G.R.A.N. (2001) for dry mater (DM), crude protein (CP), crude fibre (CF), crude fat, nitrogen free extract, ash and organic matter. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed sequentially (Van Soest *et al*., 1991) with a thermostable amylase pre-treatment. Starch was determined by the enzymatic method according to Salomonsson *et al*. (1984). For macro- and micro-elements analysis the samples were ashed at 550 °C, the ash was dissolved in 10 ml of HCl (1:3) and minerals were determined by the atomic absorption spectrometry (AAS) method, phosphorus content was determined by molybdovanadate reagent on Camspec M501. Mineralized samples were analysed for Ca, P, Mg, Na, K, Fe, Zn, Cu and Mn content. For mineral content determination the spectrometer AAS iCE 3000 (Thermo, UK) was used.

Contents of mineral nutrients in feeds and faeces were estimated in graphite cuvette through electrothermal atomization. Content of Ca was estimated at the wave length of 422.7 nm, Mg at 285.2 nm, Na at 589.0 nm, K at 766.5 nm, Fe at 248.3 nm, Zn at 213.9 nm, Cu at 324.8 nm, Mn at 279.5 nm and content of P at 410.0 nm as phosphomolybdenic yellow (Official Journal L 206, 29/07/1978, p.0043-0055). Each estimation was done in three replications. The nutrient digestibility was calculated according to following formula:

% D = (Intake – Faecal Excretion) / Intake x 100

Results were evaluated by statistical method such as significance of differences, the analysis of variance, one-way ANOVA and *t-test*, which were performed at P level less than 0.05.

Parameters in caecum

The caecal samples from each of three slaughtered rabbits (on the 91st day of age) were collected for biochemical analysis. VFA, pH, ammonia-N and lactic acid were determined; pH was measured immediately after sampling using a digital pH meter, VFA concentration was determined using gas chromatography on a 1.8 m glass column with 10 % SP1200 and 1 % H_3PO_4 on Chromosorbe WAW 80/100 mesh with isokaprylic acid as an internal standard (GC Carlo Erba). Ammonia-N concentration was measured by the micro diffusion according to Conway (Voigt and Steger, 1967). Lactic acid levels were determined by gas chromatography.

Statistical analysis

The statistical analysis was performed for all monitored traits. A linear model and a one-way analysis of variance were used for data analysis. Least square mean estimates with standard errors of the estimates were created. Differences among least square means were estimated and tested using the Tukey-test. The statistical package SAS 9.1 (SAS, 2003) was used for the analysis.

RESULTS AND DISCUSSION

The trial was carried out from July to August 2014 in the experimental house of the NAFC – Research Institute for Animal Production Nitra, Slovak Republic. The effects of dietary zinc supplementation with inorganic or organic substances on digestibility of nutrients and caecal fermentation pattern in rabbits were the priority of this study. Experimental animals did not show any health problems during the whole study period. Feeding was performed using balanced mixed feed according to feeding standards. The ability to discriminate among

diets varying in Zn concentration has been described for several animal species and nutrients. Zn is important for the organism and has influence on the feed intake; however, there is a lack of data whether rabbits can discriminate among diets differing in mineral content to avoid Zn-deficiency. The contribution of crude fibre is optimized up to the level of 14 – 16 % in rabbit mixture (NRC, 1980). Feeds were prepared at the beginning of the trial and stored at ambient temperature until they were provided to the rabbits. The samples of individual feeds were analyzed for content of nutrients, macro and micro elements (Table 1) according to AOAC (2000). Feed analyses were performed in triplicates. The content of digestible energy was calculated by the equation of Wiseman *et al*. (1992).

The chemical composition of feed gives an indication of the potential nutrient supply, but determination of digestibility provides an estimate of the nutrients available to the animal. Higher proportion of Zn in the mixture had influence on increase of in digestibility coefficients ($P > 0.05$) compared to control group. We did not find large differences among experimental groups in digestibility coefficients of starch, N-Free Extract, organic matter and Ca, P, Mg, and Cu using the balance method ($P > 0.05$) compared to the control or those fed with 100 Zn $mg.kg^{-1}$ supplemented diets. Pascual *et al*. (2008) recorded different coefficients of digestibility for dry matter, organic matter and gross energy ($P < 0.05$) between two different groups of rabbit does selected for litter size and longevity. Al-Dobain (2010) followed the effect of the diet on digestibility of four rabbit breeds. The author observed that all digestibility coefficients were significantly ($P < 0.01$) affected by the interaction dietary treatments × genetic groups. Increasing the supplemental Zn level to 100 $mg.kg^{-1}$ diet resulted in significant increase

Table 1: Content of nutrients and energy value in pelleted feed mixtures (kg per original matter)

Chemical analysis	Unit	C	1EG	2EG	3EG
		Control- C	– with $ZnSO_4.H_2O$	– with Glycinoplex- Zn	– with Bioplex- Zn
Dry matter	g	903.78	896.00	901.13	894.31
Crude protein	g	176.23	173.09	175.09	174.52
Fat	g	35.81	37.68	37.96	37.91
Crude fibre	g	158.49	157.87	146.52	145.24
ADF	g	177.62	179.41	179.08	184.58
NDF	g	338.15	311.65	312.09	311.69
Starch	g	138.94	136.58	134.61	138.94
N-Free Extract	g	461.30	458.12	472.21	461.30
Hemicellulose	g	160.87	160.87	160.87	160.87
Cellulose	g	134.57	134.57	134.57	135.34
Ash	g	71.94	71.94	71.94	71.94
Organic matter)	g	831.84	831.84	831.77	831.84
Calcium (Ca)	g	7.68	7.63	7.58	7.28
Phosphorus (P)	g	6.94	6.96	6.95	7.01
Magnesium (Mg)	g	2.64	2.63	2.73	2.71
Sodium (Na)	g	1.64	1.64	1.67	1.43
Potassium (K)	g	11.75	10.78	11.06	11.06
Iron (Fe)	mg	342.98	415.22	373.04	457.27
Zinc (Zn)	mg	126.38	246.47	246.47	257.27
Copper (Cu)	mg	20.34	21.15	22.53	22.73
Manganese (Mn)	mg	164.67	164.67	164.67	164.67
ME	$MJ.kg^{-1}$	11.07	10.94	11.37	11.43

*Premix contains per kg: calcium 6.73 g; phosphorous 4.13 g; magnesium 1.90 g; sodium 1.36 g; potassium,11.21 g; iron 0.36 g; copper 0.03 g; selenium 0.2 mg. Vitamin mixture provided per kg of diet: Vitamin A 1500000 IU; Vitamin D3 125000 IU; Vitamin E, 5000 mg; Vitamin B1, 100 mg; Vitamin B2, 500 mg; Vitamin B6, 200 mg; Vitamin B12, 0.01 mg; Vitamin K3, 0.5 mg; biotin,10 mg; folic acid, 25 mg; nicotinic acid, 4000 mg, choline chloride, 100000 mg.

of digestibility coefficients for Na, K, Fe, Mn ($P < 0.05$) and Zn ($P < 0.01$) compared to the control group. Abd El-Rahim *et al.* (1995) found that dietary supplementation of rabbit diet with 170 Zn $mg.kg^{-1}$ diet improved live body weight gain and feed conversion ratio. The digestibility of nutrients in different rabbit genotypes was studied by several authors (De Blas and Wiseman, 2010; Ondruška *et al*., 2010).

Kustos and Hullár (1992) investigated the heritability of digestibility in NZW rabbits. In their experiment, the authors determined low ($h^2 = 0.19$) heritability values for the coefficients of digestibility. Lebas (1973; 1990) in the NZW breed determined 4 % better coefficients of digestibility for dry matter and organic matter than in the Californian rabbits; these coefficients of digestibility are in concert to our results. Rafay *et al.* (2009) and Maertens and Lebas (1989) specified these values of digestibility of basic nutrients: crude protein – 75 %, crude fat – 65 % and crude fibre – 20 %.

Digestibility coefficients for crude protein and crude fibre in our experiment were higher than published by Tůmová *et al*. (2004), and Ondruška *et al*. (2011). These authors carried out a balance experiment on meat rabbits and their digestibility values of presented nutrients were 77.2 % vs. 72.6 % (crude protein) and 10.7 % vs. 15.7 % (crude fibre). The effect of dietary zinc supplementation with inorganic or organic substances on the nutrient digestibility is presented in Table 2. The digestibility coefficients for crude protein were in the range from 77.72 % to 78.77 %, which was similar to the data of Battaglini and Grandi (1988). The values of crude fibre digestibility (15.23 % – 20.64 %) and crude fat (86.70 % – 89.26 %) were higher in comparison to those of Bielański and Niedźwiadek (1993). The values of zinc digestibility (3.35 % – 19.84 %) were lower in comparison to other herbivore species, e. g. goats.

Similar relationships between the minerals are also observed in Cu deficiency, but they are less pronounced, which means that the absorption of Cu increases with Zn deficiency but that the converse is not true (Memisi *et al*., 2014). Different relationships between mineral absorption were observed with the goats received bentonite, which increased the absorption of Fe but has decreased absorption of Cu and Zn (Schwarz and Werner, 1987 Siegert *et al*., 1986). Nessrin *et al*. (2012) studied response of growing rabbits to different supplemental

Table 2: Coefficient of nutrient digestibility in % (Mean ± SD)

Item (n = 4)	Control (C)	1EC – with $ZnSO_4.H_2O$	2EC – with Glycinoplex- Zn	3EC – with Bioplex- Zn	t-test
Dry matter	63.63 ± 1.82	63.12 ± 1.54	62.50 ± 1.43	63.14 ± 0.61	n.s.
Crude protein	78.50 ± 2.24	77.16 ± 0.67	78.02 ± 1.67	78.77 ± 1.04	n.s.
Fat	86.70 ± 1.61	89.26 ± 0.64	88.42 ± 2.84	89.20 ± 1.04	n.s.
Crude fibre	18.98 ± 2.81	20.64 ± 2.44	18.87 ± 2.99	15.24 ± 1.31	$a:d^{+};b:d^{++}$
ADF	22.02 ± 2.24	19.70 ± 3.77	20.09 ± 4.36	25.06 ± 1.21	$a:d^{+}$
NDF	35.33 ± 1.69	31.14 ± 2.22	39.61 ± 4.01	28.01 ± 2.80	$a:b,c,d^{+}$
Starch	91.83 ± 0.90	94.75 ± 0.89	93.74 ± 1.34	93.50 ± 0.62	n.s.
N-Free Extract	73.31 ± 1.88	72.42 ± 1.71	71.51 ± 1.61	72.28 ± 0.74	n.s.
Hemicellulose	50.06 ± 1.41	46.66 ± 3.12	42.40 ± 5.35	34.91 ± 5.18	$a:d^{++}$; $b:d^{+}$
Cellulose	24.74 ± 2.46	21.50 ± 2.74	23.21 ± 5.27	27.13 ± 2.54	$a:b^{+};b:d^{+}$
Ash	51.15 ± 1.75	49.10 ± 1.39	47.53 ± 0.91	48.35 ± 1.70	$a:c^{+}$
Organic matter	64.02 ± 1.57	64.30 ± 1.15	63.75 ± 1.58	64.39 ± 0.59	n.s.
Calcium (Ca)	43.32 ± 5.59	53.89 ± 3.35	50.70 ± 4.14	49.02 ± 3.66	n.s.
Phosphorus (P)	32.06 ± 6.83	30.00 ± 1.80	30.15 ± 3.01	29.02 ± 3.01	n.s.
Magnesium(Mg)	25.57 ± 3.37	27.26 ± 0.69	26.30 ± 1.68	30.16 ± 3.01	n.s.
Sodium (Na)	86.29 ± 4.95	84.18 ± 4.23	70.53 ± 6.07	87.84 ± 9.91	$a:c^{+}$
Potassium (K)	87.10 ± 3.73	86.82 ± 0.55	88.95 ± 0.84	89.45 ± 0.97	$b:d^{+}$
Iron (Fe)	37.50 ± 9.77	49.73 ± 0.77	40.88 ± 3.73	40.89 ± 3.73	$b:d^{+}$
Zinc (Zn)	3.35 ± 1.37	11.10 ± 2.07	19.84 ± 3.49	15.43 ± 1.72	$a:c,d^{++}$
Copper (Cu)	17.74 ± 5.15	14.93 ± 7.17	13.00 ± 4.37	16.04 ± 5.05	n.s.
Manganese (Mn)	22.17 ± 4.27	24.85 ± 1.96	27.58 ± 2.38	32.51 ± 2.10	$a:d^{+}$; $b:d^{+}$

Control-C; 1EG – with $ZnSO_4.H_2O$; 2EG – with Glycinoplex-Zn; 3EG – with Bioplex-Zn; n.s. = $P > 0.05$; $^{+} = P \leq 0.05$; $^{++} = P \leq 0.05$

levels of zinc, magnesium or iron by following the growth performance and some carcass traits. Results of their work showed that Zn supplementation at levels of 100 or 200 mg.kg^{-1} diet significantly ($p < 0.05$) improved live weight gain and feed conversion ratio compared to the higher level of the diet (400 mg.kg^{-1}).

Absorption of zinc occurs throughout the small intestine, usually in ranges from 5 % to 40 % for intake. Transfer of zinc out of the intestinal mucosal cells to the plasma is regulated by metallothionein. Zinc absorption is reduced whenever diets are high in calcium or phytate.

Studies worldwide have shown that in some countries, regarding the presence of certain minerals in the soil, there are different variations in terms of their deficit and surplus (Anke *et al*. 1988, 1993).

Ayyat and Marai (2000) reported that supplementing rabbits with 100, 200 or 300 Zn mg.kg^{-1} significantly increased live weight gains, but had no effect on feed intake, feed conversion ratio or dressing percentage of the rabbits compared with the control or those fed 400 Zn mg.kg^{-1} supplemented diets.

Generally we can conclude that the Zn concentrations (dose of 100 mg Zn.kg^{-1} supplemented diets) used in this study have weak and/or do not have negative effect on feed intake, feed conversion ratio or dressing percentage of the rabbits compared with the control group (Table 4).

The rabbits were slaughtered before morning feeding for observation of fermentation processes in the caecum. There were no significant differences in pH value between control and experimental groups. Concentration of observed VFA shows, that the most intensive process was in the caecum of rabbits in the control group. The lower concentration of ammonia- N affects pH value in the control and experimental groups (Hoover and Heitmann, 1975). There were no significant differences ($P > 0.05$) between

Table 3: Qualitative parameters in caecum

Parameters (n = 6)	Control (C)	1EG – with $ZnSO_4.H_2O$	2EG – with Glycinoplex- Zn	3EG – with Bioplex- Zn
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
pH	5.85 ± 0.32	6.21 ± 0.18	6.07 ± 0.36	6.09 ± 0.12
N-NH3 (mmol.l^{-1})	14.36 ± 7.587	13.04 ± 3.30	12.93 ± 5.35	12.55 ± 3.56
Acetate (mmol.100 g^{-1})	7.039 ± 0.925	5.844 ± 0.651	6.853 ± 1.247	6.358 ± 1.322
Propionate (mmol.100 g^{-1})	0.569 ± 0.211	0.619 ± 0.076	0.550 ± 0.134	0.479 ± 0.036
Butyrate (mmol.100 g^{-1})	2.260 ± 1.380	1.541 ± 0.876	2.060 ± 1.293	1.589 ± 0.872
Other VFA (mmol.100 g^{-1})	0.513 ± 0.388	0.298 ± 0.129	0.284 ± 0.161	0.230 ± 0.132
Lactic acid (g.100 g^{-1})	0.0094 ± 0.003	0.0096 ± 0.001	0.0088 ± 0.003	0.0071 ± 0.005

n.s. = P > 0.05

Table 4: Growth performance of rabbits in response to dietary supplementation with zinc from inorganic or organic sources ($\bar{x} \pm SD$)

Parameters (n = 24)	Control (C)	1EG – with $ZnSO_4.H_2O$	2EG – with Glycinoplex- Zn	3EG – with Bioplex- Zn
Initial weight in g	1637 ± 119	1633 ± 33	1663 ± 183	1638 ± 93
Final weight in g	2971 ± 160	3004 ± 229	3049 ± 207	2954 ± 189
Daily weight gain in g.day^{-1}	31.76	32.63	33.00	31.34
Feed conversion ratio in g.g^{-1}	4.23	4.08	4.20	4.26
Carcass yield in %	59.24 ± 0.78	59.41 ±1.59	60.12 ± 0.45	58.37 ± 3.38

n.s. = P > 0.05

the observed rabbits, as well as between the control group and the treatments (Table 3). High lactic acid concentration was in the caecum of rabbits with the supplemented $ZnSO_4.H_2O$. These results are in agreement with those observed by many authors (Yoshida *et al*., 1972; Hossain and Bertechini, 1993; Colina *et al*., 2001). Crude fibre is digested by a microbial fermentation and main place of this fermentation is caecum (Volek *et al*., 2005). No significant effect of the diet was detected on both caecum relative weights of its content, as well as on dry matter content. Before experimental period the animals were found in good health conditions.

CONCLUSION

The current experiment was conducted to evaluate the effect of two zinc products (Glycinoplex-Zn and Bioplex-Zn), supplemented to diet, on digestibility of basic nutrients and on fermentation processes in the caecum of broiler rabbits. This study demonstrated that rabbits are able to distinguish between diets differing in Zn-content and that Zn- deficiency causes a possibly learned preference for Zn. However, the ability to avoid Zn-deficiency by feed selection seems to be influenced by several factors. However, it could be concluded that growing rabbit is tolerable to excessive dietary doses of the macroelements or Zn.

Also, a supplemental Zn in the rate of 100 $mg.kg^{-1}$ diet leads to improving live body weight gain and significantly improves feed conversion ratio of the rabbit. Additionally, the environmental impact of zinc can be reduced.

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IN VITRO GAS PRODUCTION AND DRY MATTER DEGRADATION OF FOUR BROWSE LEAVES USING CATTLE, SHEEP AND GOAT INOCULA

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ABSTRACT

The effects of rumen inoculum from Bunaji cattle, West African Dwarf (WAD) sheep and WAD goat on the in vitro gas production and dry matter (DM) degradation of *Moringa oleifera, Millettia griffoniana, Enterolobium cyclocarpum* and *Gmelina arborea* leaves were compared in an *in vitro* study using incubation periods ranging from 0 – 48 h. Oven-dried samples of the leaves were incubated in three replicates with each inoculum source and incubations run at two consecutive times to make six replicates per treatment for estimation of the kinetics of gas production using non-linear equation. Leave samples were analyzed for crude protein (CP), lignin (ADL), acid (ADF) and neutral (NDF) detergent fibres. Concentrations of CP (165 – 247 $g.kg^{-1}$ DM), NDF (413 – 538 $g.kg^{-1}$ DM) and ADF (300 – 346 $g.kg^{-1}$ DM) differed among species. Inoculum sources varied ($P < 0.05$) in volume of gas production at 12 and 24 h along the incubation but not at later incubation times of 36 and 48 h. Gas production between cattle, sheep and goat were correlated ($r = 0.98$; $P < 0.001$). Kinetics of gas production differed ($P < 0.05$) among inoculum sources with cattle inoculum showing a shorter ($P < 0.05$) lag time and higher ($P < 0.05$) rate of fermentation. Gas production also varied ($P < 0.05$) among browse species with *M. oleifera* recording the highest volume of production. *M. oleifera* and *E. cyclocarpum* were higher ($P < 0.05$) in dry matter degradation than *M. griffoniana* and *G. arborea* irrespective of inoculum source. Results indicated that *in vitro* gas production and dry matter degradation of the forages varied due to browse species and not inoculum source. Rumen fluid from cattle, sheep and goats could therefore, serve as inoculum source for the screening of these forages for ruminants.

Key words: browse species; *in vitro* degradation; cattle; sheep; goat

INTRODUCTION

In vitro techniques in feed evaluation for ruminants has gained wider acceptance due to its ease of adoption, repeatability, minimized use of animals and the decrease in funding for *in vivo* evaluation of feeds (Getachew *et al.*, 2005). Although these techniques are more rapid and precise, requiring less substrate than *in situ* procedures, they still require an inoculum to create the fermentative environment (Mould *et al.*, 2005). Different ruminant species including cattle, sheep, goats, deer, chamois and buffalo have been used as inoculum donors. Small ruminants especially sheep and goat are commonly used as donor animals for *in vitro* trials in most African countries, more particularly in Nigeria (Abegunde *et al*., 2009; Anele *et al*., 2009; Ajayi and Babayemi, 2008; Babayemi and Bamikole, 2009), probably due to ease of animal handling, reduced cost of maintenance and lower feed requirements relative to larger ruminants such as cattle (Bueno *et al*., 2005).

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Several studies have reported the comparative ability of different ruminant species to digest fibrous feeds, fodder trees and shrubs (Larbi *et al*., 1997; Bueno *et al.*, 1999; Cone *et al*., 2000; Calabro *et al.*, 2004; Bueno *et al.*, 2005; Ammar *et al.*, 2008). Most studies made comparisons only between two ruminant species and quantitative data is limited to *in vitro* digestibility of shrub fodder among West African indigenous breeds of cattle, sheep and goats. This study compared *in vitro* gas production and dry matter (DM) degradation of foliage from four tropical browse species using inoculum from Bunaji cattle (BUC), West African Dwarf (WAD) sheep and WAD goats. This is an attempt to ascertain if rumen fluid from any of the three donors could accurately serve as inoculum for *in vitro* testing to predict the digestibility of these feeds for ruminants.

MATERIALS AND METHODS

Experimental design

A 3 (inoculum source) × 4 (browse species) factorial arrangement in a randomized complete block design was used in this study.

Collection of browse species

Fresh leaves plus edible twigs of *Moringa oleifera*, *Millettia griffoniana*, *Enterolobium cyclocarpum* and *Gmelina arborea* were harvested in triplicates from mature trees at the experimental plot of the College of Animal Science and Livestock Production, University of Agriculture, Abeokuta, southwestern Nigeria in May 2010. The region has a humid climate with a mean annual rainfall of 1037 mm; and mean annual temperature and humidity are 34.7 °C and 82 %, respectively. Freshly harvested foliage from each species was sub-sampled and initially weighed fresh on the field and then oven-dried at 65 °C to constant weight. Dried samples were weighed and hammer-milled to pass through a 1mm sieve and then stored for subsequent analysis.

Chemical analysis of browse leaves

Total nitrogen (N) was determined by the Kjeldahl method (AOAC, 1990; ID 973.18). Crude protein (CP) was then calculated as N x 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest *et al.* (1991). Neutral detergent fibre was determined without amylase and sodium sulphite. Both NDF and ADF were both exclusive of residual ash. Lignin was determined by solubilisation of cellulose with sulphuric acid on the ADF residue (Van Soest *et al*. (1991).

Animal donors and collection of rumen fluid

Two 350 kg BUC, three WAD sheep (45 kg average weight) and three WAD goats (35 kg average weight) were used as inoculum donors. The animals were previously fed with 600 $g.kg^{-1}$ DM of *Pennisetum purpureum* and 400 $g.kg^{-1}$ DM of concentrate diet. The concentrate consisted of (as fed basis, $g.kg^{-1}$) 400 corn, 100 wheat offal, 100 palm kernel cake, 200 groundnut cake, 50 meal, 100 dried brewers grain, 10 common salt, 37.5 oyster shell and 2.5 fish meal. Rumen fluid was collected in equal proportions from the donor animals, under the same feeding regime within 15 min before the morning meal into thermo flasks and strained through four-layered cheesecloth and kept at 39 °C soon after collection. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. The rumen liquor and a buffer solution were mixed in the ratio 1:2 (v/v), respectively as described by Menke and Steingass (1988).

***In vitro* procedure**

Incubation was carried out at 39 °C and the volume of gas production was measured at 3 h interval from 3 to 48 h using procedures described by Menke and Steingass (1988). Three blanks containing 30 ml of medium only were included in the run. Average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. Gas volumes obtained at varying incubation hours were fitted to the non-linear equation model of France *et al*. (2002):

$$A = b\,(1-e^{-c(t-L)})$$

where: **A** is the volume of gas produced at time *t*, *b* is the asymptotic/potential gas production (ml/g DM) from the fermentable fraction of forage, *c* is the fractional rate of gas production (/h) from the slowly fermentable feed fraction *b*, and *L* is the discrete lag time prior to gas production. Two runs of incubations were performed consecutively resulting in six replicates per treatment.

***In vitro* dry matter degradation**

The *in vitro* dry matter degradation was determined at 48 h by centrifuging the incubation residues at 20, 000 x g for 30 min following placement in iced cubed (-4 °C) to end fermentation. Residues obtained were filtered and oven-dried to determine their dry weight. The blanks were also centrifuged and residues weighed and used to correct for residues from the ruminal inoculum. *In vitro* dry matter degradation was then calculated as:

$$\frac{\text{Substrate dry matter incubated} - (\text{residue dry matter} - \text{blank dry matter})}{\text{Substrate dry matter incubated}}$$

Gas production ratios

The ratio of cumulative gas production at 24 h and 48 h (GP24/GP48) were compared in an attempt to ascertain how much of the fermentation was completed in the first 24 h (Bueno *et al.*, 2005). Similarly, the ratio of 48 h cumulative gas production and asymptotic gas production, *b* (GP48/b) were compared in order to determine how close 48 h gas production is from *b*. The closer 48 h gas production is to *b* (i.e. higher ratio), the better the feed quality and/or the incubation time was long enough to express the fermentation potential of the feed.

Statistical analyses

Data were analyzed as a 3 x 4 factorial arrangement in a randomized complete block design using the general linear models (GLM) procedure of SAS (1999) with the model:

$$Y_{ijk} = \mu + B_i + R_j + \beta n + (BR)_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the observation, μ is the population mean, B_i is the browse species effect ($i = 1 – 4$), R_j is the inoculum source (cattle, sheep, goat) effect ($j = 1 – 3$), βn is the block effect (repeated incubation; n = 1 – 2), $(BR)_{ij}$ is the interaction between browse species and inoculum source and ε_{ijk} is the residual error. Regression analysis was used to

Table 1: Chemical composition of leaves of browse species

Browse species	Composition				
	[a]DM	[b]CP	[c]NDF	[d]ADF	[e]ADL
Moringa oleifera	245	247	413	300	79
Millettia griffoniana	345	165	537	346	84
Enterolobium cyclocarpum	344	182	514	319	86
Gmelina arborea	372	179	538	349	88
Mean	326	193	501	329	84
SEM	14.98	9.59	15.76	7.06	1.11

[a]DM: dry matter (g.kg^{-1} as fed basis), [b]CP: crude protein (g.kg DM^{-1}), [c]NDF: neutral detergent fibre (g.kg DM^{-1}), [d]ADF: acid detergent fibre (g.kg DM^{-1}), [e]ADL: acid detergent lignin (g.kg DM^{-1}).

Table 2: Cumulative gas production using rumen fluid from Bunaji cattle, and West African Dwarf sheep and goats as inoculum

Browse species	GP-12 h[a]			GP-24 h[b]			GP-36 h[c]			GP-48 h[d]		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Moringa oleifera	143	133	133	168	153	158	188	183	187	205	198	197
Millettia griffoniana	93	85	90	115	113	107	143	140	145	160	160	167
Enterolobium cyclocarpum	127	120	120	153	137	138	170	167	172	190	183	188
Gmelina arborea	97	83	82	125	110	113	145	143	140	170	168	167
SEM												
Browse (B)		2.10			3.46			2.25			1.94	
Inoculum source (R)		6.54			6.52			5.90			4.79	
B*R		3.74			3.78			3.32			2.71	
P > F												
B		< 0.001			< 0.001			< 0.001			< 0.001	
R		0.0002			0.0138			ns			ns	
B*R		ns			ns			ns			ns	

[a]GP-12 h: cumulative gas production after 12 h of incubation (ml.g DM^{-1}), [b]GP-24 h: cumulative gas production after 24 h of incubation (ml.g DM^{-1}), [c]GP-36 h: cumulative gas production after 36 h of incubation (ml.g DM^{-1}), [d]GP-48 h: cumulative gas production after 48 h of incubation (ml.g DM^{-1}), [e]ns: not significant.

establish relationships between gas production using cattle, sheep and goat rumen fluid. Means were separated with Fisher's Least Significant Difference (SAS, 1999) when $P \leq 0.05$.

RESULTS

Chemical composition of browse leaves

Crude protein concentration was highest in the leaves of *M. oleifera* and lowest in *M. griffoniana* (Table 1). The cell wall concentration was highest in *G. arborea* and lowest in *M. oleifera*.

Cumulative gas production

Cumulative gas volumes varied ($P < 0.05$) among inoculum sources at 12 and 24 h of incubation when gas volumes measured were higher with feeds incubated with cattle rumen fluid than those measured with sheep and goat rumen fluid (Table 2). Cumulative gas volumes with cattle rumen fluid were relatively higher than using the rumen fluid of sheep and goats (Fig. 1). Browse species affected ($P < 0.05$) cumulative gas production (Table 2). Gas production from *M. oleifera* was higher than those of the other browse species at all incubation periods in cattle, sheep and goats (Fig. 2 a, b, c).

Kinetics of gas production

Potential gas production was significantly affected by browse species, with potential gas production of *M. oleifera* and *E.cyclocarpum* being higher than *M. griffoniana* and *G. arborea* (Table 3). The rate of fermentation varied ($P < 0.05$) among browse species and source of inoculum (Table 3). *M. oleifera* had relatively higher rate of fermentation than *M. griffoniana*, *E.cyclocarpum* and *G. arborea*; while rate of fermentation using cattle rumen fluid was higher than sheep and goats. The browse species*source of inoculum interaction significantly affected the lag time (Table 3). *Gmelina* had the highest lag time with cattle and goat rumen fluid, while *Millettia* had the highest lag time with sheep rumen fluid. Cattle rumen fluid had shorter ($P < 0.05$) lag times than sheep and goat rumen fluid.

Ratio of GP24 to GP48 and GP48 to *b*

The ratio of cumulative gas production at 24 h to that of 48 h (GP24/GP48) was influenced ($P < 0.05$) by the browse species and source of inoculum (Table 4). The GP24/GP48 using cattle rumen fluid was higher than sheep and goats; while *M. oleifera* and *E. cyclocarpum* had higher ($P < 0.05$) ratio than *M. griffoniana* and *G. arborea*.

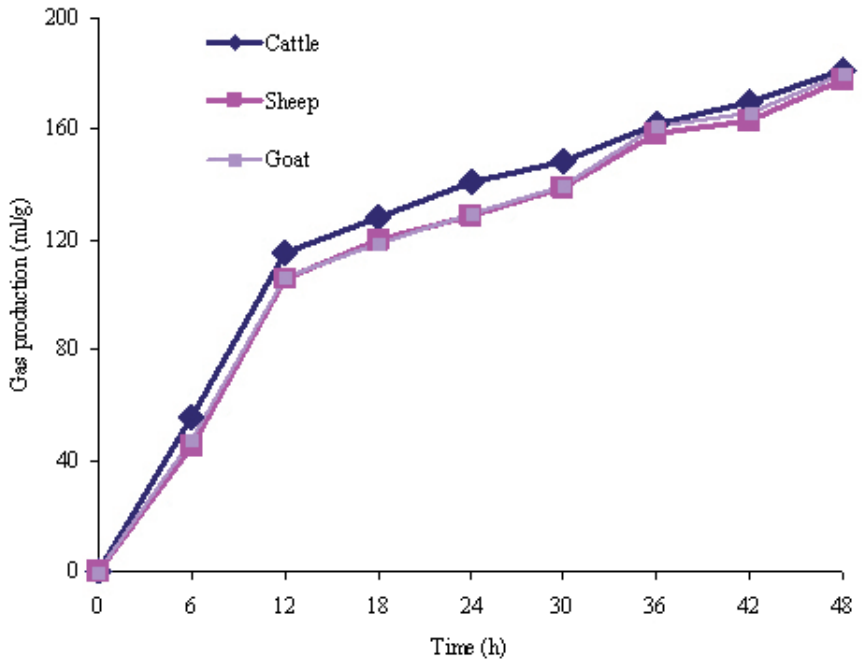

Fig. 1: Gas production from fermentation of leaves of four browse species at varying incubation times using cattle, sheep and goat rumen fluid as inoculum

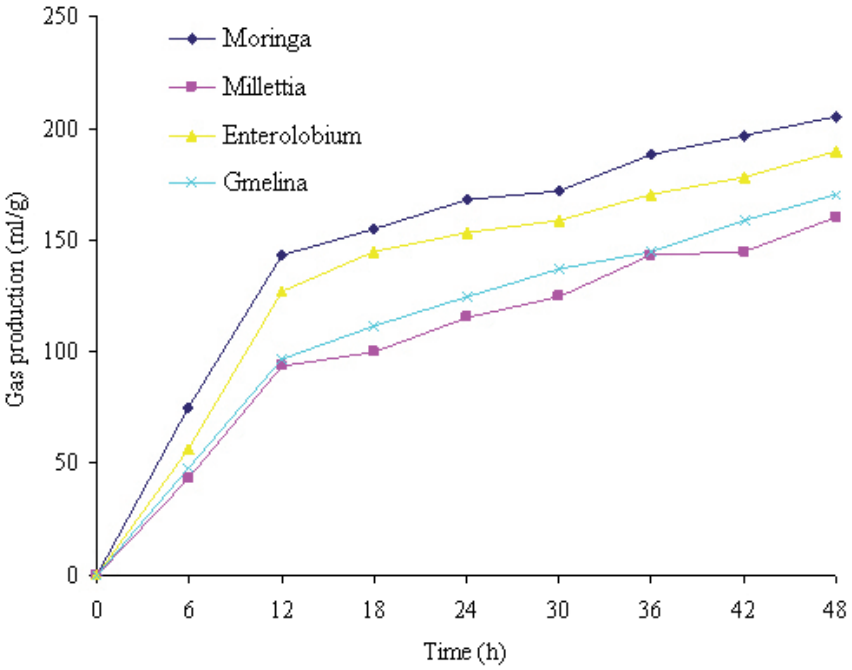

Fig. 2a: ***In vitro*** **gas production profile of four browse species at varying incubation times using rumen fluid from Bunaji cattle**

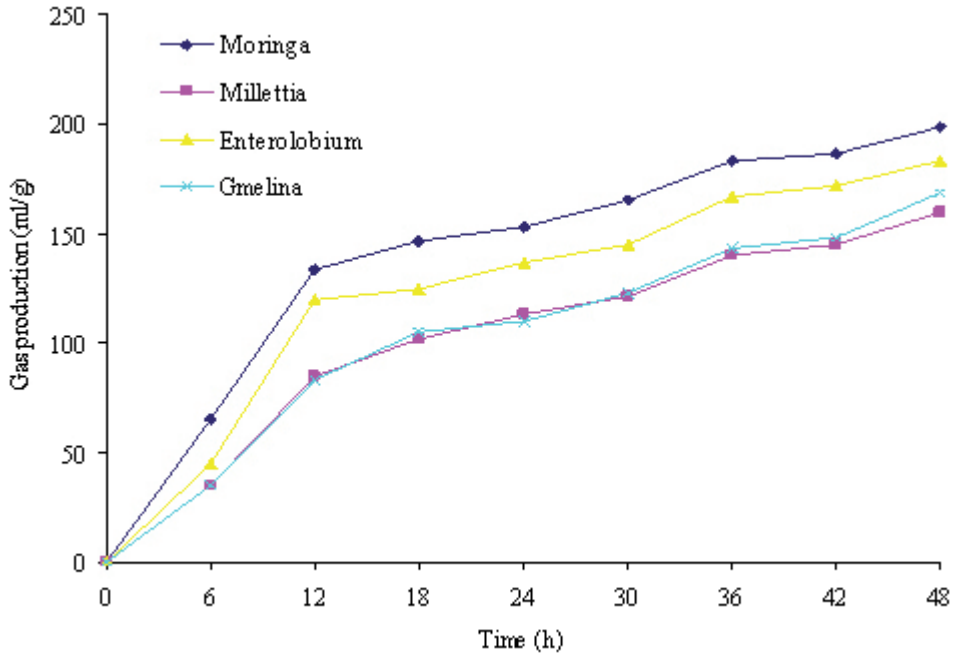

Fig. 2b: ***In vitro*** **gas production of four browse species at varying incubation times using rumen fluid from West African dwarf sheep**

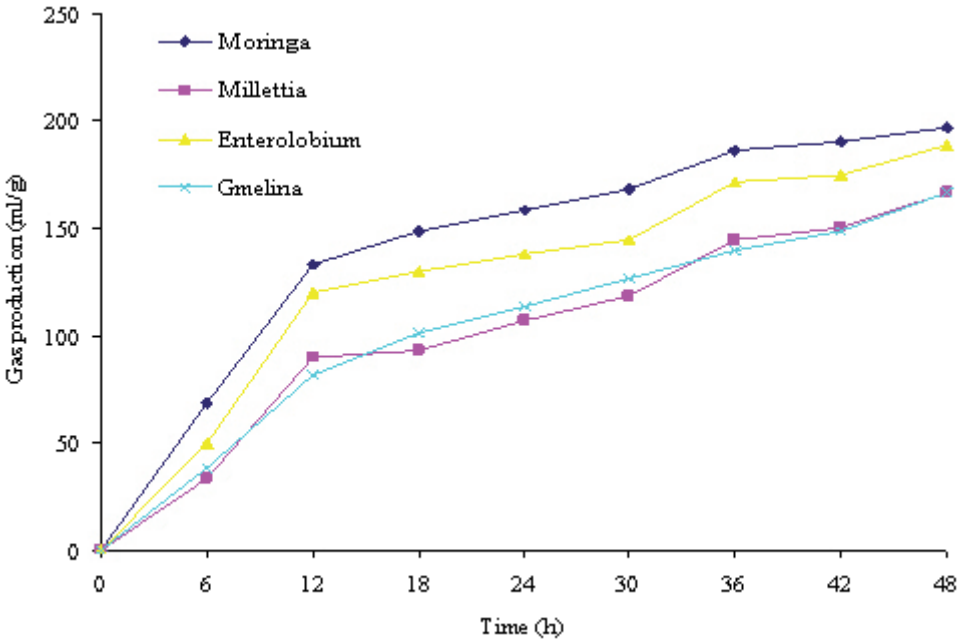

Fig. 2c: ***In vitro*** **gas production of four browse species at varying incubation times using rumen fluid from West African dwarf Goat**

Table 3: Gas production kinetics of browse leaves using rumen fluid from cattle, sheep, and goats

Browse species	*b*[a]			*c*[b]			lag time[c]		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Moringa. oleifera	210	201	201	0.073	0.062	0.067	1.10	1.16	1.14
Millettia griffoniana	165	163	172	0.060	0.050	0.051	1.00	1.24	1.08
Enterolobium cyclocarpum	193	189	191	0.058	0.051	0.054	1.06	1.20	1.20
Gmelina. arborea	176	172	170	0.058	0.047	0.056	1.18	1.22	1.22
SEM									
Browse (B)		2.09			0.0026			0.0250	
Inoculum source (R)		4.73			0.0025			0.0199	
B*R		2.68			0.0016			0.0140	
P > F									
B		< 0.0001			0.0014			0.0028	
R		ns			0.0147			< 0.0001	
B*R		ns			ns			0.0194	

[a]*b*: asymptotic gas production from insoluble fraction (ml.g DM^{-1}; France *et al*., 2002); [b]*c*: rate of gas production (/h);
[c]lag time (h); ns: not significant

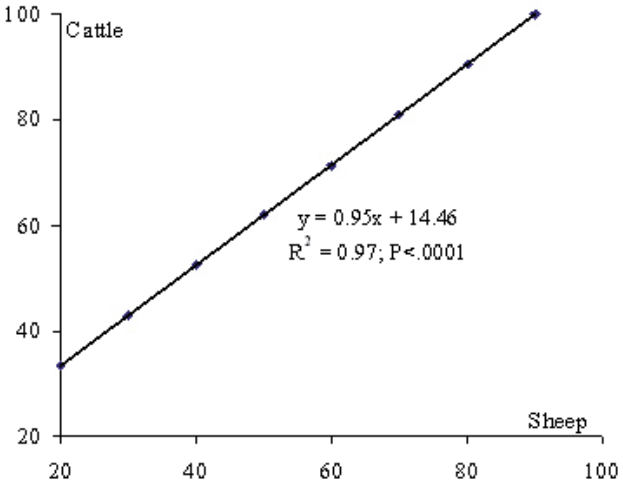

Fig. 3a: Relationships between *in vitro* cumulative gas production using cattle and sheep inoculum

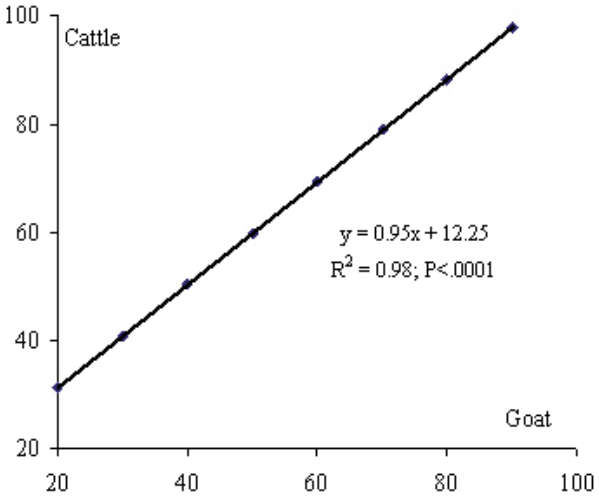

Fig. 3b: Relationships between *in vitro* cumulative gas production using cattle and goat inoculum

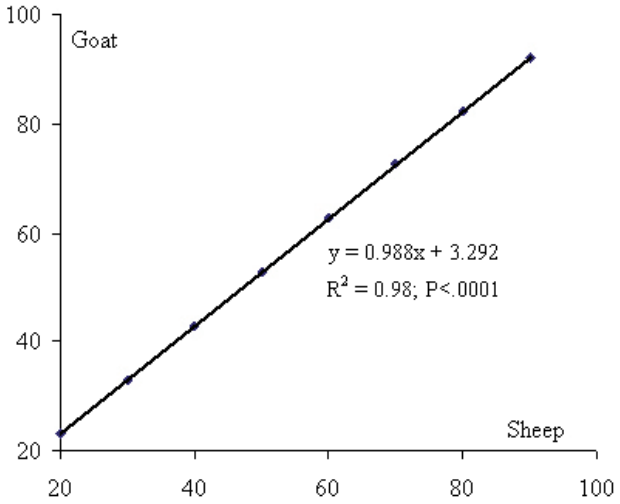

Fig. 3c: Relationships between *in vitro* cumulative gas production using goat and sheep inoculum

Relationship between gas production using cattle, sheep and goat inocula

As shown in Fig. 3a, 3b, 3c, cumulative gas production at 48 h from cattle and sheep inocula, cattle and goat inocula, as well as goat and sheep inocula were significant and highly correlated ($r = 0.98$; $P < 0.001$) .

Dry matter degradability

Browse species significantly affected *in vitro* DM degradation (IVDMD), with degradation of *M. oleifera* and *E. cyclocarpum* being higher than *M. griffoniana* and *G. arborea* (Table 5).

DISCUSSION

Chemical composition

Chemical composition varied among browse species in support of earlier studies with shrubs and trees (Ammar *et al.*, 2004b; Camacho *et al.*, 2010; Larbi *et al.*, 1997; Larbi *et al.*, 2011; Mbugua *et al.*, 2008; Salem *et al.*, 2007). Variations in CP and cell wall content could possibly be due to differences in ratio of leaves and twigs in forages as similarly reported by Larbi *et al.* (2011). The mean CP (193 g.kg DM^{-1}) and NDF (501 g.kg DM^{-1}) values for browse species in this study are consistent with earlier reports for browse species in the tropics (Anele *et al.*, 2009; Larbi *et al.*, 1998). The CP of browses was above 80 g.kg DM^{-1}, the reported minimum required in diet for adequate digestive activities of rumen microbes (Orskov, 1982). The high CP content in these species is an advantage to rumen microbes that depend on dietary source of nitrogen to build up their body proteins.

Table 4: Ratio between cumulative gas production at 24 h and 48 h incubation (GP24/GP48) and ratio between gas production at 48 h and *b* (GP48/b) of browse leaves using rumen fluid from cattle, sheep and goat

Browse species	GP24/GP48			GP48/*b*		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Moringa oleifera	0.821	0.773	0.807	0.977	0.989	0.979
Millettia griffoniana	0.720	0.709	0.642	0.971	0.980	0.968
Enterolobium cyclocarpum	0.807	0.746	0.734	0.985	0.968	0.986
Gmelina arborea	0.734	0.651	0.679	0.965	0.979	0.980
Means	0.770	0.720	0.715	0.974	0.979	0.978
SEM						
Browse species (B)		0.018			0.005	
Inoculum source (R)		0.020			0.004	
B*R		0.012			0.003	
P > F						
B		0.0002			ns	
R		0.0302			ns	
B*R		ns			ns	

ns: not significant

Table 5: *In vitro* dry matter degradation (g.kg DM^{-1}) of browse leaves using rumen fluid from cattle, sheep, and goat[$]

Browse species	IVDMD[a]		
	Cattle	Sheep	Goat
Moringa. oleifera	617	603	610
Millettia griffoniana	511	503	505
Enterolobium cyclocarpum	558	536	559
Gmelina arborea	521	507	512
SEM			
Browse species (B)		10.54	
Inoculum source (R)		15.30	
B*R		8.69	
P > F			
B		< 0.0001	
R		ns	
B*R		ns	

[a]IVDMD: *in vitro* dry matter degradation; ns: not significant

Cumulative gas production

The variation in cumulative gas production among inoculum sources at 12 h and 24 h incubation period could possibly be due to variation in microbial activity such as a shorter lag time and higher rate of fermentation with cattle rumen fluid than with either sheep or goat rumen fluid (Table 3). Comparable with our results, variation in gas production at intermediate times along the incubation periods were reported when feeds were incubated with sheep and buffalo rumen fluids (Calabro *et al.*, 2005), as well as at earlier incubation times with cattle and sheep rumen fluid (Bueno *et al*., 2005). Our results suggest that variation may occur along the incubation period but differences among inoculums would be insignificant

at end incubation hours, in agreement with earlier studies (Bueno *et al*., 2005; Calabro *et al*., 2005).

Cumulative gas production volumes varied among browse species. Differences in chemical composition (i.e. CP and NDF) (Table 1), differences in morphological composition (i.e. leaf, stem), as well as reported concentrations of anti-nutritional components such as tannin (Larbi *et al.*, 2011; Ndijja and Nasiru, 2010; Rittner and Reed, 1992) could be responsible. A higher gas volume among browse species corresponded to a higher CP and lower cell wall content. Positive correlation between crude protein and gas production in browse species have been reported (Gasmi-Boubaker *et al*., 2005; Ndlovu and Nherera, 1997).

The browse species followed similar trend in extent of gas production with cattle, sheep and goat rumen fluid (Fig. 2a, b, c). This observation implies that the browse species were ranked similarly with cattle, sheep and goat inocula suggesting that any of cattle, sheep or goat rumen fluid could be used in *in vitro* fermentation studies to examine differences between browse species. Coppock *et al*. (1988) and Calabro *et al*. (2005) reported similar trends when feeds were incubated with rumen fluid from some species of ruminants in a comparative study.

Kinetics of gas production

Potential gas production b did not vary among cattle, sheep or goat inoculums in agreement with earlier work by Cone *et al.* (2000) and Bueno *et al*. (2005) who reported similarity in the estimates of total gas production between inoculums collected from cattle and sheep. Variation in *b* among browse species confirm earlier works (Ammar *et al*. 2004a; Ammar and Gonzalez, 2005; Bueno *et al*., 1999), which could be due to variation in chemical composition of the browse species (Table1).

Rate of fermentation was higher and lag time shorter with cattle rumen fluid. Shorter lag time observed in our study with cattle rumen fluid is consistent with Bueno *et al*. (1999) who reported a longer lag period with sheep rumen fluid than rumen fluid from cattle. Lag time is indicative of the time taken for microbes to adhere themselves to the substrates, and microbial attachment to insoluble substrate has been reported to be a pre-condition for digestion to proceed (Kudo *et al*., 1995). The shorter lag time could be responsible for the faster rate of fermentation with cattle rumen fluid (Table 4), implying that fermentation of browse species incubated with cattle rumen fluid proceeded faster than those with sheep and goat. This could also explain the variation observed among cattle, sheep and goat inocula in cumulative gas production along the incubation period. Variation in dynamics of gas production has been reported with cattle and sheep rumen fluid (Bueno *et al*., 2005; Cone *et al*., 2000) and is indicative of the fact that one species could not be used to predict the gas production profile of the other (Bueno *et al*., 2005).

The higher extent of gas production and rate of degradation of *M. oleifera* suggests that rumen microbes were able to utilize the feed better probably due to a higher content of fermentable nutrients. A higher potential gas production can contribute significantly to energy supply via short chain fatty acid production (Remesy *et al*., 1995). The longer lag times observed for cattle and goat inocula with *G. arborea* and that observed for sheep with *M. griffoniana* could not be clearly explained but these could be responsible for the lower potential gas production of the two browse species relative to *M. oleifera* and *E. cyclocarpum.*

Ratio between gas production parameters

The ratio between GP-24 h and GP-48 h was higher with cattle rumen fluid than with sheep and goat. This is comparable with results obtained by Bueno *et al.* (2005) who reported a higher ratio with cattle rumen fluid than with sheep rumen fluid. Our findings imply that a greater extent of fermentation had taken place half time the incubation period with cattle rumen fluid, suggesting cattle rumen fluid as having a higher fermentation efficiency half time the incubation period. Sheep and goat rumen fluid could be considered as requiring extended periods of incubation for efficient substrate fermentation *in vitro*.

***In vitro* dry matter degradability**

Inoculum source did not affect IVDMD in agreement with earlier studies (Ammar *et al.,* 2004b; Dalmau *et al*., 2006; Mabjeesh *et al.*, 2000) suggesting possible similarity in microbial species and activity. Microbial population is dependent on the type of diet fed and since the three donors were maintained on the same diet, microbial species were not expected to vary. Differences between ruminant species in terms of digestive capability become noticeable only when each species are fed a different diet, and are considerably reduced when all animals receive the same diet (Ammar *et al.*, 2008; Dalmau *et al*., 2006; Mould *et al*., 2005). Several authors (Aerts *et al*., 1985; Bueno *et al*., 1999; Larbi *et al*., 1993; Tolkamp and Brouwer, 1993) suggested good agreement in the digestive capacity of cattle, sheep and goat. The often stated superiority of goats over sheep and cattle in terms of forage digestibility (Domingue *et al*., 1991) was not confirmed in this study.

The IVDMD varied with browse species possibly due to differences in cell wall content. The higher IVDMD observed in *M. oleifera* is attributed to its higher potential gas production (Table 3). Digestibility has been reported to be synonymous to *in vitro* gas production, with a high positive correlation obtained between gas production and dry matter digestibility (Datt and Singh, 1995; Fieves *et al.*, 2005).

Relationship between gas production using cattle, sheep and goat rumen fluid

Average values of gas production with cattle, sheep and goat inocula were highly correlated suggesting that rumen fluid from Bunaji cattle, WAD sheep or WAD goat could be reliable in predicting the gas production by any of the browse species. Similar to our results, significant correlations between sheep and cattle, sheep and goat or sheep and buffalo have been reported (Ammar *et al*., 2008; Bueno *et al.*, 2005; Calabro *et al*., 2005; Cone *et al*., 2000).

CONCLUSION

In conclusion, our results indicate that inoculum from cattle, sheep or goats were well correlated and can be used to determine the potential gas production and IVDMD of any of the four browse species. Browse species with higher crude protein and lower cell wall content showed better potential for gas production and in vitro dry matter digestibility. Variation in gas production and IVDMD would therefore vary due to species differences rather than inoculum source in an *in vitro* medium.

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DAIRY CALF MORBIDITY AND MORTALITY AND ASSOCIATED RISK FACTORS IN SODO TOWN AND ITS SUBURBS, WOLAITA ZONE, ETHIOPIA

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ABSTRACT

A longitudinal observational study on calf morbidity and mortality in dairy farms in Sodo town and its suburbs was conducted from January 2013 to January 2014 with the aim of investigating dairy calf morbidity and mortality rate, determining potential risk factors associated with calf morbidity and mortality, and isolating some enteropathogens associated with diarrhea. All 30 dairy calves, which were born during the period from January 2013 to June 2013 at eight dairy farms, were enrolled for the study. Patterns of the calves' morbidity and mortality were followed up from birth to the end of their sixth months of age at individual level. In addition, a questionnaire survey on calf rearing practices was performed on the farms, where the experimental animals resided. The results of this study demonstrated 66.7 % (n = 20) calf morbidity and 20 % (n = 6) mortality. Diarrhea accounted for 63.3 % (n = 19) of the morbidity, while pneumonia accounted for 3.3 % (n = 1). The main cause of death was also diarrhea resulting in three out of six deaths. Based on the laboratory examination, *Eschericia coli* only was excreted by 26.3 % (n = 5) of the diarrheic calves, *Salmonella* only by 10.5 % (n = 2), and *Cryptosporidium* by 52.6 % (n = 10); *E. coli* + *Salmonella* were concurrently excreted by 10.5 % (n = 2) of the diarrheic calves. Overall, 76.9 % (n = 20) of the 26 examined animals were found to be infected by different gastrointestinal and ectoparasites. The association of 21 potential risk factors with dairy calf morbidity and mortality was investigated. Of these factors, among others poor body condition of the dam, short teat distant from the ground, feeding calves less than four liters of milk/day, were significantly associated with dairy calf morbidity ($p < 0.05$), whereas less than five year farm work experience of herd attendants, and a stock of less than ten animals in a farm were significantly associated with dairy calf mortality ($p < 0.05$). On the other hand, manure removal once at day was significantly associated with both calf morbidity and mortality ($p < 0.05$). In conclusion, calf morbidity and mortality was found to be relatively high in the examined area, and can have short-term and long-term detrimental effects on dairy production by suppressing growth rate of the calves and replacement capacity of the herd.

Key words: dairy calf; morbidity; mortality; risk factors; *Eschericia coli*; *Salmonella*; *Cryptosporidium*

INTRODUCTION

Dairy farming is a growing livestock production system in Ethiopia. It is primary source of income for urban and peri-urban poor communities. Because of better availability of milk market, most of the dairy farms are concentrated in urban and peri-urban areas of the country. Development of market-oriented dairy farming is given high attention in the country and is growing in Sodo town and its suburbs. Farmers show considerable interest in raising dairy cows and are organizing in unions to combine their efforts and money to run dairy farms. They also increase the use of exotic dairy cattle and their crosses in order to enhance milk production. However, since exotic cattle are less tolerant to local diseases, the dairy production is facing a great challenge due to high prevalence of diseases in dairy cows (Lemma *et al*., 2001) and their offspring.

A successful dairy farm operation requires that a large percentage of cows wean a live healthy calf every year. Rearing healthy dairy calves to weaning time requires maximizing the calf's level of immunity against

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disease while minimizing its exposure to infectious agents (Godden, 2008). However, among the factors that have been hindering success of dairy industry, morbidity and mortality of calves is the one, that causes major concern (Acha *et al*., 2004). Phiri (2008) also noted that morbidity and mortality are important causes of economic losses on dairy farms worldwide. In spite of advancement made in dairy husbandry practices, clinical medicine and diagnostic techniques, the morbidity and mortality rates of dairy calves are still unacceptably high even on many advanced dairy farms in developed countries (Mee, 2008). Thus, it is necessary to identify risk factors that are responsible for dairy calf morbidity and mortality in order to design and implement preventive measures.

According to Lorenz *et al.* (2011), calf morbidity and mortality have short-term and long-term detrimental effects on performance of a dairy farm. They impair both growth rate and replacement capacity of the herd (MacGurik and Ruegg, http://www.progressivedairy.com/dairy-basics/calf-and-heifer-raising/2230-0209-pd-calf-diseases-and-prevention.referred in November 2013). Calf hood diseases have, therefore, a significant financial impact on dairies resulting from treatment costs, genetic loss, and impaired future performance (Donovan *et al*., 1998). Furthermore, many of the infectious agents that cause calf diarrhea can pose a considerable threat to humans (*E. coli*, *Salmonella*, *Campylobacter*, and *Cryptosporidium*). The prevalence of multidrug resistance among the Salmonella strains has increased over the past two decades (Kevin *et al.*, 2010) causing an increase in treatment failure and hospitalization rates (Swai *et al.*, 2010; Varma *et al.*, 2005). Thus, controlling infections caused by these microorganisms in dairy calves can provide economic, health and welfare benefits in the dairy industry and may reduce the zoonotic risk.

No investigation of dairy calf morbidity and mortality was carried out in the examined area before. Hence, large scale risk factor analysis is needed to quantify the calf morbidity and mortality as well as to identify associated risk factors. Understanding the causes of common calf morbidity and mortality, their methods of transmission, and associated risk factors in the studied area is the first step in developing effective programs to minimize their impact on calf health and thereby to reduce their threat to public health.

Therefore, the objectives of this study were: to investigate morbidity and mortality of dairy calves to the age of six months, to isolate *E.coli*, *Salmonella*, and *Cryptosporidium* from affected calves, to identify other parasites that affect the calves from the collected feces, and to explore risk factors associated with calf morbidity and mortality in order to give effective advice and realistic recommendations to farmers.

MATERIAL AND METHODS

Experimental animals

All 30 calves born during the period from January 2013 to June 2013 were enrolled for the study. A longitudinal observational study was conducted on 30 dairy calves at eight dairy farms that are distributed throughout Sodo town and its suburbs. Patterns of the calves' morbidity and mortality were followed up from birth to the end of their sixth months of age at individual level. Upon enrollment, the calves were given identification number and their date of birth, sex, breed, presence of delivery complications like dystocia, retained placenta and colostrum delivery strategy to the calves were recorded. In addition the dam's parity, breed, udder condition, health and milk yield were recorded. Body condition score of the dams was determined (Radostits *et al.*, 2007) and recorded at the late pregnancy during the farm visiting time by the first investigator. The farms, where the examined animals resided, were visited six times during the study period by the investigators. These visits enabled us to observe the health status of the calves. During each visit the calves management, housing and sanitation situations were also observed. Health issues of the calves occurred out of the visiting time were communicated by the owners with the investigators. The sick animals were treated after the clinical investigation and necessary samples were collected.

Sampling techniques

Fecal sampling for parasite examination

Fecal samples were collected in June and August 2013 from 26 dairy calves at eight farms. Four calves died before the fecal sample collection. The samples were collected directly from the recta of the calves with surgical gloves and were placed in clean dry screw-cup universal bottles. Each specimen was clearly labeled and transported to the laboratory of School of Veterinary Medicine, Wolaita Sodo University in an ice box at a temperature about +4 °C. In the laboratory, the samples were examined for the presence of parasites on the day of collection. Standard floatation and sedimentation methods were used in the diagnosis of parasitism in the examined animals (MAFF, 1979).

Fecal sampling for bacterial cultures and isolation

Fecal samples from 19 diarrheic calves were collected on the day of onset of diarrhea. A calf was considered as diarrheic if feces was semi-fluid (loose) to fluid with or without mucus and/or blood. About 50 grams of feces were collected directly from the rectum of affected animals with a sterile latex surgical glove

(HLL Lifecare Limited, Kerala, India) and placed into a sterile screw-cup universal bottle. Each specimen was clearly labeled and transported to the Wolaita Sodo Regional Laboratory at an ice cold condition for bacterial culture.

Samples were intended to examination for the presence of *E. coli* strain K99, *Salmonella*, and *Cryptosporidium*. However, due to the problem encountered to secure the ELISA kit for *E. coli* strain K99, *E. coli* was isolated only at the species level. The bacteriological examination was carried out according to standard methods of Quinn *et al*. (2002).

Detection of *Cryptosporidium*

Fecal samples were also examined for the presence of *Cryptosporidium*. This was done by modified Zeihl-Nelson staining technique, as described by Kaufmann (1996). Identification of oocysts from smears was made by comparing with the slide photograph in Kaufmann (1996). A smear was considered positive, when one or more oocysts were observed.

Identification of other calf diseases

Prevalence of other calf diseases was diagnosed by clinical examination of affected animal.

Questionnaire survey

In this study, a survey on calf rearing practices that might be involved in dairy calves' morbidity and mortality was performed on the farms, where the experimental animals resided. The farms are distributed throughout Sodo town and Sodo suburb. The questionnaire survey was designed to provide data on critical factors that might be associated with morbidity and mortality of dairy calves at the farm level. The on-farm survey included a face-to-face personal interview with the farm manager or senior worker by the investigators using a standard questionnaire (Wudu *et al*., 2008). The questionnaire was developed consisting multiple choice (yes or no) and semi-closed questions. We added some questions and modified others. The questionnaire was divided into nine groupings of management practices that could affect dairy calf health: herd size, farm, care of the newborn, calf-dam separation, calf housing, calf feeding, weaning and health constraints. The answers to the questions (data) were qualitative nominal (e.g., yes or no), or continuous (e.g. once in a day, number of liters of milk). The collected data were verified and validated at the same time on the farm. In addition the repeated visits to the farms permitted us to verify and validate the data collected via the questioner survey.

Data management and statistical analysis

Processing of data was done by a computer software. All the collected data were stored, filtered in Microsoft excel spread sheet and transferred to SPSS version 20.0 for analysis. P- value < 0.05 was considered as significant. Prevalence of calf morbidity and mortality (the dependent variable) and independent variables, such as farm attributes, farm management factors, calf management factors both at herd level and calf level were considered. The association between dependent and independent variables was tested using Chi-square test. The responses of all variables were dichotomized to facilitate analysis and interpretation of results. While dichotomizing continuous variables and those categorical variables with response of more than two levels, a care was taken to make the cutoff points sensible.

RESULTS

The prevalence of diarrhea in the examined dairy calves during the follow up period was 63.3 % (n = 19) and respiratory (pneumonia) disease - 3.3 % (n = 1). The mortality found during the examination period was 20 % (n = 6). Of the diarrheic dairy calves, 68.4 % (n = 13) were affected at the age of less than two months, followed by 26.3 % (n = 5) at the age of 2–4 months, and 5.3 % (n = 1) at the age of 4–6 months. *Escherichia coli* only was excreted by 26.3 % (n = 5) of the diarrheic dairy calves, *Salmonella* only by 10.5 % (n = 2), and Cryptosporidium by 52.6 % (n = 10). Concurrent infection with two microorganisms (*E.coli* + *Salmonella*) occurred in 10.5 % (n = 2) of diarrheic dairy calves

Table 1: Bacterial and Cryptosporidium isolates

Age (month)	No. of calf (%)	*E. coli*	*Salmonella*	*Cryptosporidium*	Concurrent infection *E. coli* + *Salmonella*
< 2	13 (68.4)	4	1	6	2
2–4	5 (26.3)	1	1	3	0
4–6	1 (5.3)	0	0	1	0

(Table 1). The main cause of death was diarrhea accounting three out of six deaths. The three dairy calves showed classical clinical signs like profuse watery diarrhea, dehydration, weakness, depression and recumbence before death. Two calves died suddenly showing clinical signs of heart water while one calf died of pneumonia.

The parasite infestation of the examined animals is shown in Table 2. Four calves of the examined animals died before fecal sample for parasite investigation was collected. Overall, 76.9 % (n = 20) of the 26 examined animals were found to be infected by different parasites. *Trichostrongylus* was the parasite that affected large number of calves (65.4 %, n = 17, five of them were concurrently infected with other parasites.) followed by *Trichuris* (15.4 %, n = 4, all of them concurrently infected with other parasites), *Eimeria* (7.7 %, n = 2), and *Neoascaris vitulorum* (3.8 %, n = 1 concurrently infected with other parasites). Concurrent infestation with two or more parasites occurred in 23.1 % (n = 5) of the affected calves (Table 2). The peak parasite infestation of dairy calves occurred at the age of 2–4 months; 7.7 % of the calves (n = 2) were identified with skin lesions caused by ectoparasites (Table 2).

Factors related to dams associated with calf morbidity and mortality are shown in Table 3 and 4, respectively. Poor body condition of the dam was significantly ($p < 0.05$) linked to high morbidity and (not significantly) to increased mortality in dairy calves (83.3 %, n = 15/18 and 22.2 %, n = 4/18, respectively). Multiparity of cows accounted for significantly increased ($p < 0.05$) morbidity and slightly higher mortality (75.0 %, n = 18/24 and 20.8 %, n = 5/24, respectively) in dairy calves compared to the influence created by the primiparity of the dams. Despite morbidity and mortality in dairy calves, born from dams affected by dystocia and retained placenta, were high, they did not significantly differ from the rate of morbidity and mortality in calves born from cows without parturition problems. Significantly higher ($p < 0.05$) morbidity and non-significant but more mortality were attributed to less than 50 cm dams' teat distant from the ground (91.7 %, n = 11/12 and 25 %, n = 4/16, respectively). Increased morbidity (80.0 %, n = 8/10) and higher ($p = 0.053$) mortality (30.0 %, n = 3/10) were also observed in calves born from cows with large teats.

Factors related to calf associated with calf morbidity and mortality are shown in Tables 5 and 6. Higher morbidity (77.8 %, n = 14/18) and mortality (22.2 %, n = 4/18) were registered in calves below three months of age compared to 50 % (n = 6/12) and 16.7 % (n = 2/12), respectively in calves above three months of age. More female dairy calves (71.4 %, n = 15/21) were affected by diarrhea than male calves (55.6 %, 5/9), while relatively high percentage of male calves died (22.2 %, n = 2/9), than female calves (19 %, n = 4/21).

Association of herd level farm management practice with calf morbidity and mortality is summarized in Tables 7 and 8. There was higher morbidity (80.0 %, n = 4/5) and significantly more ($p < 0.05$) mortality of calves (60.0 %, n = 3/5) reared in farms having less than 10 animals, than in calves reared in farms having 10 or more animals (60.0 %, n = 15/25 and 12.0 %, n = 3/25, respectively). Hundred percent of dairy calves reared on mud floor were sick, compared to calves kept on a concrete floor (100 %, n = 6/6 and 20.8 %, n = 5/24, respectively). Significantly higher ($p < 0.05$) mortality was registered in farms where calves were reared by herd attendants having less than five years of dairy farm work experience (Tables 7 and 8). Morbidity of dairy calves cared by only male herd attendants was significantly higher ($p < 0.05$) than calves reared by female and male workers (88.9 %, n = 16/18 and 33.3 %, n = 4/12, respectively). Mortality

Table 2: Parasite infestation of the dairy calves

Age (month)	No. of calf (%)	*Trichostrongylus* only	*Trichuris* only	*Neoascaris Vitulorum* only	*Eimeria* only	Ectoparasite only	No. of concurrently infested Calves
1–2	3 (11.6)	0	0	0	2	1	
2–4	11 (50.0)	7	0	0	0	0	4*
4–6	6 (38.5)	5	0	0	0	0	1**

*Calf 1 was infested by *Trichostrongylus* + *Trichuris* + *Neoascaris vitulorum*. Calves 2 and 3 were infested by *Trichostrongylus* + *Trichuris*, Calf 4 was infested by *Trichostrongylus* + Ectoparasite

**One calf was infected by *Trichostrongylus* + *Trichuris*

Table 3: Factors related to dam associated with dairy calf morbidity

Variable (Dam)	Description	No. of calves	No. of sick calves (%)	X^2	df	p-value
Body condition	Poor	18	15 (83.3)	0.000	1	0.043
	Moderate	12	5 (41.7)			
Parity	Multiparous	24	18 (75.0)	0.000	1	0.027
	Primiparous	6	2 (33.3)			
Teat distant from the ground	≥ 50 cm	18	9 (50.0)	5.625	1	0.018
	< 50 cm	12	11 (91.7)			
Teat size	Large	10	8 (80.0)	1.200	1	0.273
	Normal	20	12 (60.0)			
Dystocia	Yes	9	7 (77.8)	0.714	1	0.398
	No	21	13 (61.9)			
Retained placenta	Yes	7	6 (87.5)	1.491	1	0.222
	No	23	14 (60.9)			

X^2 = Chi-square df = Degree of freedom Significant at $p < 0.05$

Table 4: Factors related to dam associated with calf mortality

Variable (Dam)	Description	No. of calves	No. of died calves (%)	X^2	df	p-value
				0.000	1	0.043
Body condition	Poor	18	4 (22.2)	0.139	1	0.709
	Moderate	12	2 (1.7)			
Parity	Multiparous	24	5 (20.8)	0.052	1	0.819
	Primiparous	6	1 (16.7)			
Teat distant from the ground	≥ 50 cm	14	2 (14.2)	1.875	1	0.171
	< 50 cm	16	4 (25.0)			
Teat size	Large	10	3 (30.0)	3.750	1	0.053
	Normal	20	3 (15.0)			
Dystocia	Yes	4	2 (50.0)	2.960	1	1.107
	No	26	4 (15.4)			
Retained placenta	Yes	4	2 (50.0)	2.596	1	0.107
	No	26	4 (15.4)			

X^2 = Chi-square df = Degree of freedom Significant at $p < 0.05$

Table 5: Factors related to calf associated with calf morbidity

Variable (Calves)	Description	No. of calves	No. of sick calves (%)	X^2	df	p-value
Age	< 3 months	18 (60.0)	14 (77.8)	2.500	1	0.114
	≥ 3months	12 (40.0)	6 (50.0)			
Sex	Male	9 (30.0)	5 (55.6)	0.714	1	0.398
	Female	21 (70.0)	15 (71.4)			

Table 6: Association of factors related to calf with calf mortality

Variable (Calves)	Description	No. of calves	No. of calves died (%)	X^2	df	p-value
Age	< 3 months	18	4 (22.2)	0.139	1	0.709
	≥ 3months	12	2 (16.7)			
Sex	Male	9	2 (22.2)	0.635	1	0.426
	Female	21	4 (19.0)			

Table 7: Association of herd level farm management practice with calf morbidity

Variables	Description	No. of calf (No. of sick calf)	Morbidity rate	X^2	df	p=value
Herd size	≥ 10 animals	25 (16)	64.0	0.718	1	0.397
	< 10 animals	5 (4)	80.0			
Floor of farm	Concrete	24 (14)	58.3	1.292	1	0.256
	Mud	6 (6)	100.0			
Farm work experience of herd attendants	< 5 years	19 (14)	73.7	1.148	1	0.284
	≥ 5 years	11 (6)	54.5			
Sex of herd attendants	Male	18 (16)	88.9	0.577	1	0.002
	Male and female	12 (4)	33.3			

Table 8: Association of herd level farm management practice with calf mortality

Variables	Description	No. of calf (No. of calf died)	Mortality rate	X^2	df	p=value
Herd size	≥ 10 animals	25 (3)	12.0	6.000	1	0.041
	< 10 animals	5 (3)	60.0			
Floor of farm	Concrete	24 (5)	20.8	0.052	1	0.819
	Mud	6 (1)	16.7			
Farm work experience of herd attendants	< 5 years	19 (6)	31.6	4.342	1	0.037
	≥ 5 years	11 (0)	0.0			
Sex of herd attendants	Male	18 (4)	22.2	0.139	1	0.709
	Male and female	12 (2)	16.7			

was also moderately higher in the same farms (22.2 %, n = 4/18).

Association of calf feeding practice with dairy calf morbidity and mortality is presented in Tables 9 and 10. Delayed first colostrum feeding led to increased morbidity and mortality (80.0 %, n = 8/10 and 40.0 %, n = 4/10, respectively) compared to early colostrum consumption (60.0 %, n = 12/20 and 10.0 %, n = 2/20, respectively). High morbidity and mortality were evidenced in dairy calves fed milk from the pool (76.5 %, n = 13/17 and 29.4 %, n = 5/17, respectively) compared to calves fed milk collected individually from their dams (53.8 %, n = 7/13 and 7.7 %, n = 1/13, respectively). Morbidity in calves fed less than four liters of milk

per day was significantly high ($p < 0.05$) compared with calves fed four liters and above milk per day (92.9 %, n = 13/14; 43.8 %. n = 7/16, respectively). Although not significant, mortality was also high in calves fed less than four liters of milk per day (21.4 %, n = 3/14). In this study, limited water supply of calves was found to be associated with increased morbidity and relatively low mortality (77.3 %, n = 17/22 and 18.2 %, n = 4/22, respectively), whereas *ad libitum* water supply was associated with low morbidity and higher mortality (37.5 %, n = 3/8 and 25.0 %, n = 2/8, respectively).

The association of herd level calving management and care of the newborn with calf morbidity and mortality is indicated in Tables 11 and 12. More calves that were born in the tie-stall were sick and dead (69.2 %, n = 9/13 and 30.8 %, n = 4/13, respectively) than calves born in calving pen (64.7 %, n = 11/17 and 11.8 %, n = 2/17, respectively). Increased morbidity was recorded in navel untreated calves compared to navel treated calves (70.8 %, n = 17/24 and 50.0 %, n = 3/6, respectively). Differencing from calf morbidity, significantly high percentage ($p < 0.05$) of calves with disinfected navels at birth died compared to calves without disinfected navels (50.0 %, n = 3/6; 12.5 %, n = 3/24, respectively). It was also noted that manure removal frequency significantly influenced dairy calf mortality ($p < 0.05$) and morbidity ($p < 0.01$) (31.6 %, n = 6/19 and 89.5 %, n = 17/19, respectively). All of the calves died among the examined animals belonged to the herds, where manure was removed only once a day. The current study revealed that mortality was higher (30.8 % (n = 4/13) in calves from farms with calf stocking space of less than 1.6 meter square per calf (< 1.6 m²/calf) whereas morbidity in the same stocking was slightly higher (69.2 %, n = 9/13).

Table 9: Association of herd level calf feeding practice with calf morbidity

Variables	Description	No. of calf (No. of sick calf)	Morbidity rate	X^2	df	p=value
First colostrum feeding time	> 6 hrs	10 (8)	80.0	1.200	1	0.273
	≤ 6 hrs	20 (12)	60.0			
Source of milk	Pool	17 (13)	76.5	1.697	1	0.193
	The dam	13 (7)	53.8			
Amount of milk fed/day	< 4 liters	14 (13)	92.9	8.103	1	0.004
	≥ 4 liters	16 (7)	43.8			
Water supply	*Ad libitum*	8 (3)	37.5	2.131	1	0.144
	Limited	22 (17)	77.3			

Table 10: Association of herd level calf feeding practice with calf mortality

Variables	Description	No. of calf (No. of sick calf)	Mortality rate	X^2	df	p=value
First colostrum feeding time	> 6 hrs	10 (4)	40.0	3.750	1	0.584
	≤ 6 hrs	20 (2)	10.0			
Source of milk	Pool	17 (5)	29.4	2.171	1	0.141
	The dam	13 (1)	7.7			
Amount of milk fed/day	< 4 liters	14 (3)	21.4	0.033	1	0.855
	≥ 4 liters	16 (3)	18.8			
Water supply	*Ad libitum*	8 (2)	25.0	0.170	1	0.680
	Limited	22 (4)	18.2			

Table 11: Association of herd level calving management and care of the newborn with calf morbidity

Variables	Description	No. of calf (No. of sick calf)	Morbidity rate	X^2	df	p=value
Calving location	Calving pen	17 (11)	64.7	0.068	1	0.794
	At the tie-stall	13 (9)	69.2			
Navel treatment	Practiced	6 (3)	50.0	0.938	1	0.333
	Not practiced	24 (17)	70.8			
Manure removal	Once/day	19 (17)	89.5	12.129	1	0.000
	> Once/day	11 (3)	27.3			
Calf stocking	< 1.6 m²	13 (9)	69.2	0.068	1	0.197
	≥ 1.6 m²	17 (11)	64.7			

Table 12: Association of herd level calving management and care of the newborn with calf mortality

Variables	Description	No. of calf (No. of calf died)	Mortality rate	X^2	df	p=value
Calving location	Calving pen	17 (2)	11.8	1.663	1	0.197
	At the tie-stall	13 (4)	30.8			
Navel treatment	Practiced	6 (3)	50.0	4.219	1	0.040
	Not practiced	24 (3)	12.5			
Manure removal	Once/day	19 (6)	31.6	4.342	1	0.037
	> Once/day	11 (0)	0.00			
Calf stocking	< 1.6 m²/calf	13 (4)	30.8	1.663	1	0.197
	≥ 1.6 m²/calf	17 (2)	11.8			

DISCUSSION

This study attempted to determine dairy calf morbidity and mortality, identifying the importance and magnitude of the factors that put dairy calves at risk of morbidity and mortality, and isolating some of the pathogenic agents that caused diarrhea in the calves.

The overall morbidity and mortality recorded in this study were 66.7 % and 20 %, respectively. This result is very close to the findings of Wudu *et al.* (2008), where the crude dairy calf morbidity was 62.0 % and mortality 22.0 %. Although diarrhea was the most important cause of morbidity in both studies, the prevalence of diarrhea in this study was higher (63.3 %) than the incidence of diarrhea indicated by Wudu *et al*. (2008) (42.9 %). The outcome of this study confirmed the finding of Gulliksen *et al.* (2009) that diarrhea is the most frequent health disorder of calves. Very low morbidity of calves was attributed to respiratory disease (3.3 %) in this study compared to the findings of McGurik (2008) (25 %). Wudu *et al*. (2008) also found 4.9 % incidence of calf pneumonia, which is very close to the result of this study. The low incidence of calf pneumonia in the present study may be because cases of pneumonia were not detected by the animal keepers. Identification of early signs of calf pneumonia depends on good observational skills of the herd attendants. As Sivula *et al.* (1996) have shown in their study, animal keeper diagnosis of pneumonia is only 56 % sensitive. The high incidence of diarrhea (78.9 %) among the diarrheic dairy calves occurred at the age of less than two months. This may be because newborn calves at their early age are highly susceptible to diarrhea causing agents. According to McGurik and Ruegg, (http://www.progressivedairy.com/dairy-basics/calf-and-heifer-raising/2230-0209-pd-calf-diseases-and-prevention. referred in November 2013), the highest morbidity and mortality rates generally occur in baby calves prior to weaning. The calf mortality (20 %) rate registered

in this investigation is consistent with the findings of Swai *et al.* (2010) (21 %) and Wudu *et al.* (2008) (22 %), and higher than 8.4 % by McGuric (2008), 16.81 % by Bangar, *et al.* (2013). On the other hand, it was less than the 25.0 % mortality indicated by Sisay and Ebro (1998) and the 50 % by Hassan (1996) in Ethiopia. The relatively less mortality rate in our study compared with the findings of Sisay and Ebro (1998) and Hassan (1996) was probably due to current better access to veterinary service in towns and their suburbs. In addition, the low number of calves reared in each farm of this study coupled with the extension work of the development agents might made the sick calf management relatively better.

From the 19 fecal samples collected from the nineteen diarrheic calves, five (26.3 %) were *E.coli* only positive, followed by two (10.5 %) *Salmonella* only positive, and ten (52.6 %) *Cryptosporidium* positive. Concurrent infection with two microorganisms (*E.coli* + *Salmonella*) occurred in 10.5 % (n = 2) of diarrheic dairy calves. Serotyping of the isolated *E. coli* was not performed due to failure of securing a kit. *E. coli* can be isolated from healthy calves and adult cows as well as calves with diarrhea. It can be normal intestinal flora. This creates uncertainty if the *E. coli* recovered from the samples is causative to the disease. However, based on the clinical signs shown by the diarrheic calves, situation of the dairy calves' environment, and as the disease is self-limiting, it may be possible to come to conclusion that the most likely cause of the diarrhea problem is *E. coli.* Unless there is an outbreak, *E. coli* can be isolated at species level for simple diagnosis purpose.

Overall 76.9 % of the 26 examined calves were found to be infected by different parasites. Peak parasite infestation occurred at the age of two to four months. This was probably the age when calves start to consume increased amount of grass and are naive to parasite infestation. In addition the calves that were reared at the suburbs of Sodo were allowed to graze around the farms where adult cows were grazing. The rest of the calves consume grass bought from the market. Thus, probably the calves acquired infective larval stages of parasites from the grass they consumed. The calves might be infected by *Eimeria* and *Cryptosporidium* when they ingest infective oocysts from the manure-contaminated environment.

A total of 21 different potential risk factors were assessed to determine the magnitude of their association to occurrence of dairy calf morbidity and mortality in the followed up farms. Morbidity in calves fed less than four liters of milk per day was significantly ($p < 0.05$) higher compared to calves fed four and above liters of milk per day. Although not significant, calf mortality was also higher in calves fed less than four liters of milk per day. Milk is an excellent source of nutrition providing large amounts of crude protein, energy, vitamins and minerals for the calves at their early ages, which are essential among others to maintain efficacy of their body defense mechanism. Feeding a calf less than four liters of milk per day mainly at its early age, when it does not consume solid feed, was below standard. A study by Rincker *et al.* (2006) showed benefits of feeding calves larger amounts of milk than the traditional 10 to 12 % body weight per day. Those calves that were fed less than four liters of milk per day probably suffered hunger. The restricted diet and the stress caused by the hunger might have immunosuppressive effect making them vulnerable to diseases.

Higher morbidity ($p = 0.273$) and mortality ($p = 0.053$) were revealed in calves born from dams having large teats. This might be because calves faced difficulty in handling the large teats resulting in consumption of inadequate quantity of colostrum. All the calves in the monitored dairy farms were allowed to remain with their mother after birth and suckle colostrum without interference. Morin *et al.* (2010) warned that there is no guarantee that calves will have a sufficient intake by leaving them to suckle colostrum without interference. Vasseur *et al*. (2009) also stated that poor colostrum intake caused high morbidity and mortality in dairy calves. Therefore, in this case low colostrum intake probably caused morbidity and mortality of the calves. Furthermore, significantly ($p < 0.05$) high morbidity and more mortality ($p = 0.171$) were recorded in calves born to dams having udders hanged too low less than 50 cm teat distant from the ground. Consequently, the calves probably had trouble of finding the teats and pushing the heavy udder upward in order to be able to suckle colostrum ending up in consuming inadequate quantity of colostrum.

Dystocia and retained placenta were found to be other risk factors associated with morbidity and mortality of calves. Quigley (1997) noted that calves that were born from cows with dystocia have a higher mortality. Newborn calves stressed due to dystocia are weak enough to adapt to life in the external environment. This stress to the calves probably reduced the immunoglobulin absorption efficiency as well as delayed or decreased intake of colostrum. Hence, the longer calves are without adequate colostrum Ig, the more opportunity for the pathogens that provoke diarrhea to invade the gut. According to Lombard *et al.* (2007) dystocia has been estimated to increase calf death risk by 4– to 8–fold. The investigators of this study observed that some of the dairy farmers falsely believe that feeding colostrum from dam with retained placenta will harm the calf. Thus they refrained from feeding their calves colostrum on time waiting until the retained placenta was removed.

Poor body condition of the dam at late pregnancy

was significantly ($p < 0.05$) associated with calf morbidity and insignificantly associated with increased calf mortality ($p = 0.709$). Many researchers and farmers determine the nutritional well-being of the cow by the body condition score (Weaver *et al.*, 2000). Thus, the poor body condition of the dam reflects its suffering from deficiency of energy, protein, vitamins and other nutrients. The prepartum diet affects colostrum quality (Lemma *et al.*, 2001). According to Arthington *et al*. (2000), calves that were fed colostrum, obtained from cows that were fed restricted amounts of energy and crude protein, showed reduced absorption of IgG by 21.8 %. Furthermore, Quigley (1997) declared that calves born from the dams with inadequate nutrient intake before parturition might be more susceptible to morbidity and mortality. Therefore, the total amount of immunoglobulin available to calves probably was much less than the required in the situation where the cows scored poor body condition.

The Chi-square analysis of morbidity and mortality in dairy calves with respect to parity showed that calves born from multiparous cows had significantly ($p < 0.05$) high morbidity and non-significantly high mortality ($p = 0.819$) comparing to calves born from primiparous cows. This was probably because multiparous cows are susceptible to mastitis. The susceptibility of cows varies considerably and new infections are most common in older cows at early lactation and when the management is poor. According to Sargeant *et al*. (2000) and Radostits *et al*. (2007), the risk of clinical mastitis also increases with increasing parity. Cows that are affected by either clinical or subclinical mastitis shed pathogenic micro-organisms through the milk. The calves that consumed the contaminated milk might be affected by diarrhea.

It was also noted that more female calves were sick compared to male calves, while higher mortality occurred in male calves than in female calves. More female calves were sick probably because there were more female calves among the studied animals (70 %). The other explanation to this finding could be that perhaps farmers watched female calves more carefully due to their economic importance and thus diagnosed many clinical cases more effectively. More male calves died probably because postnatal mortality for males and females had a very high genetic correlation, with direct heritability being highest for males (Hanssen *et al*., 2003). According to the findings of Swai *et al*. (2010), male animals in Tanga, Tanzania were three times more likely to die than females. Similar result was obtained by Bangar *et al.* (2013) in India.

In this investigation, less than five years farm work experience of herd attendants was found to be significantly ($p < 0.05$) associated with calf mortality and non-significantly ($p = 0.284$) associated with calf morbidity. This was probably because taking care of sick calves required more work experience than caring for healthy calves. Sex of the herd attendants was also found to significantly ($p < 0.05$) influence morbidity of dairy calves. Significantly higher ($p < 0.05$) percentage of calves was sick in herds where only male herd attendants took care of calves compared to calves cared by both male and female farm workers. This might be because of the influence of women's activity regarding to calf rearing in the working team. A woman knows better how to take care of young life.

All calves kept on mud floored farm were sick compared to calves kept on concrete floor. This might be because of difficulty in keeping mud floors clean and dry. Besides, they were also less effectively disinfected. As Lindsay (2012) stated, muddy, wet conditions have proven to be the source of increased morbidity because disease causing bacteria can grow rapidly. Significantly ($p < 0.05$) high mortality of dairy calves occurred in farms where less than ten animals were kept. The most important determining factor of whether a herd had high or low calf morbidity and mortality is the quality of calf management. Ninety percents of the surveyed farms served as the secondary source of income to the owners. Farmers working in farms with less number of animals will have much less income from the farm. Thus, they probably spent much of their time on working in other places to cover their cost of living. This probably partially diverted their attention that should have been fully paid to calves. This poor caring of sick calves might result in the significantly high mortality.

In this study, calves that had their first colostrum meal after six hours of age had experienced increased mortality and morbidity. This observation was consistent with the findings of Wudu *et al.* (2008). On farms colostrum administration practice is the primary determinant of calf health. According to the observation of the investigators some of the herd men/women in this study area wrongly believed that consumption of the first colostrum causes diarrhea in calves. As the result, they milk and discard the first colostrum before the newborn suckles. Hence, the newborn calves were probably allowed to consume colostrum late. Furthermore, according to Godden (2008) colostral immunoglobulin content is reduced with each successive milking; therefore the first milking colostrum has more immunoglobulin content than the second milking colostrum. To ensure adequate protection against disease, calves rely on the intake of an adequate amount of quality colostrum within a few hours of birth (Arthington *et al*., 2000). The ability of the neonate to absorb immunoglobulin starts to decline progressively after 6 to 12 hours from birth (Radostits *et al*., 2007). Colostral Ig concentration also decreases by 3.7 % during each subsequent hour post-calving (Morin *et al*.,

2010). Therefore, the late a calf consumes colostrum after birth, the lower the level of immunoglobulin absorption. As Arthington *et al.* (2000) noted, low blood Ig concentration is directly related to calf morbidity and mortality.

There was higher morbidity and mortality in calves delivered at the tie-stall compared with the calves born in calving pens. In 43.3 % of the farms calves were delivered at the tie-stall (Table 11) and they remain there for 24 hr or more. Under such conditions there was high chance of contamination of the udder and teats with feces and urine. Thus calves might acquire massive doses of pathogens at birth from the tie-stall floor contaminated by the manure of adult animals before they found colostrum. They might also ingest pathogens from the manure at the udder and teat during colostrum suckling. *E. coli* and *Salmonella* infection is common where sanitation is poor. The infection of intestine by pathogens suppresses absorption of immunoglobulin, which probably resulted in diarrheal disease and death of the dairy calves. The result of this study was in agreement with the finding of Svensson *et al*. (2003).

In this study, mortality was significantly high ($p < 0.05$) in calves where navel treatment was practiced, while morbidity was higher in navel untreated calves although was not statistically significant ($p = 0.333$). The increased morbidity of navel untreated calves might be attributable to the entrance of pathogens from the contaminated calves environment through the umbilicus. Navel infection is one of the disease conditions which has serious impact on the survival of calves (Wudu *et al.*, 2008). According to Quigley (1997), early disinfection accelerated drying up of the umbilicus to reduce infections so that calf respiratory and enteric diseases and mortality are decreased. Disinfection of the umbilicus did not positively influence mortality of the calves in this study.

Removal of manure from the stall only once a day was significantly associated with dairy calf morbidity and mortality ($p < 0.01$, $p < 0.05$, respectively). Manure is important source of pathogens. Accumulation of manure in the stall might contaminate calves' feed and water exposing the calves to overwhelming pathogens. Among the management risk factors investigated, cleanness of the calf house was found important (Wudu *et al*., 2008; Marce *et al*., 2010) in relation to calf mortality and morbidity. Furthermore, Phiri (2008) reported that newborn calves had a higher risk of diarrhea when stalls were not cleaned periodically. Although not significantly, calf stocking was found to be associated with high calf morbidity and mortality ($p = 0.197$ for both). This result was in accordance with the previous report by Benadli *et al.* (1999). This might occur due to increased pathogen contamination from fellow animals and posed stress.

In this study, high morbidity was demonstrated in calves fed milk from the pool and had restricted water access. On the other hand, these factors were not associated with calf mortality. Water should be provided free-choice starting at four days of age. Providing calves with water ad libitum increases solid feed intake and weight gain. In a research study, depriving calves of drinking water decreased starter intake by 31 % and decreased weight gain by 38 % over those calves provided water *ad libitum*. Proper dry matter and energy intake is critical in providing resistance to disease in young calves. In calves, a higher plane of nutrition improves immune function (Drackley, 2005) and also lowers mortality and the incidence of diarrhea (Kevin *et al*., 2010). 76.5 % of the surveyed herds in our study fed their calves milk from the pool. Weaver *et al.* (2000) and Lindsay (2012) do not recommend the use of pools because pooling milk may increase calves' exposure to milk-borne pathogens.

CONCLUSIONS

Calf morbidity and mortality was found to be relatively high in the examined area and can have short-term and long-term detrimental effects on dairy production by suppressing growth rate of the calves and replacement capacity of the herd. The association of 21 potential risk factors with dairy calf morbidity and mortality was investigated. Of these factors, poor body condition of the dam, multiparity, short teat distant from the ground, male herd attendants, once a day manure removal, less than four liter of milk consumption per day were significantly associated with dairy calf morbidity. Whereas less farm work experience of herd attendants, treated navel, once a day manure removal and a stock of less than ten animals per farm were significantly associated with dairy calf mortality. This risk factor assessment may be considered as the first step on devising an intervention strategy to prevent dairy calf morbidity and mortality and, thereby, improve dairy production in the examined area.

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