In the Name of God

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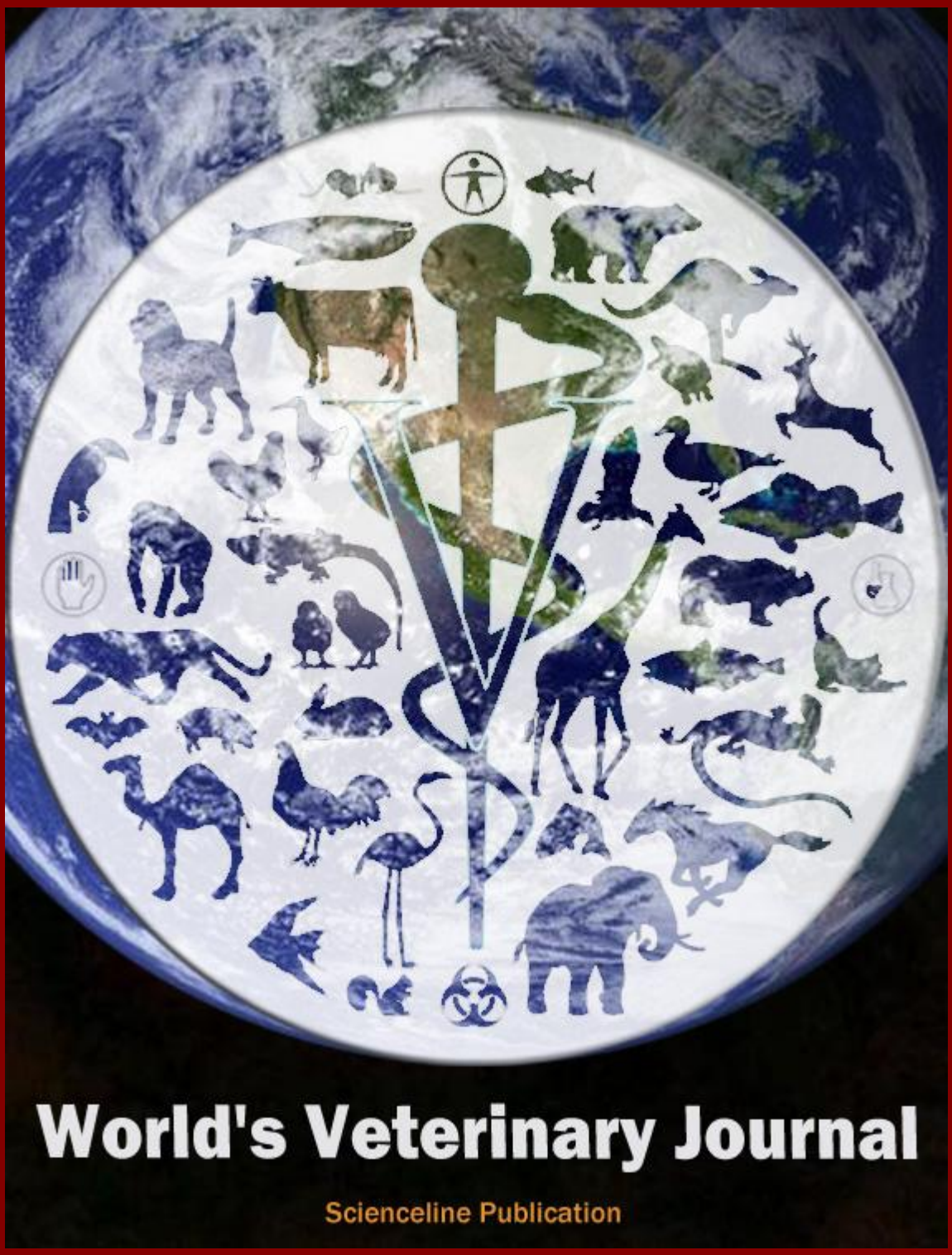

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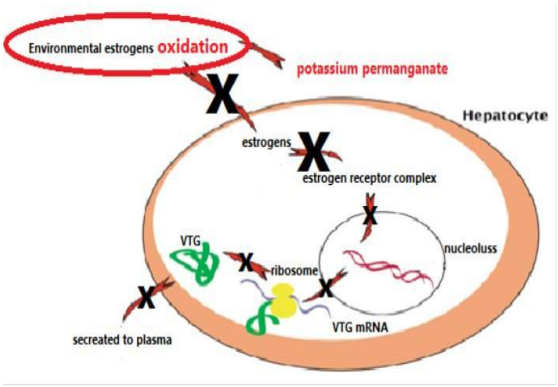
Research Paper

Potassium Permanganate Alleviates the Potential Effect of Estrogenic Pollutants on Vitellogenin Gene Expression in Male Oreochromis niloticus.

Magdy Elgaabary A, Mahmoud Sh, Fahmy Saad M, Abdel Azeez Abdel Rahman A.

World Vet. J. 6(2): 38-45, 2016; pii:S232245681600006-6



ABSTRACT

This study aimed to determine the concentration of some estrogens like 17β-estradiol (E2), 17α-ethynylestradiol (EE2) and bisphenol A (BPA) in agriculture drainage water that used in Egyptian fish farms and to evaluate the oxidative degradation effect of potassium permanganate on these estrogenic pollutants. In addition to assess vitellogenin gene expression on mature male *Oreochromis niloticus* as a biomarker for estrogenisity. Three groups of males were allocated in three farm ponds filled with agriculture drainage water used in Egyptian fish farms. Water of two of them was treated with potassium permanganate in a concentration of 2.5 and 5 ppm while that of the third pond was kept as untreated control. Other three groups were allocated in three laboratory glass aquariums filled with tap water. Water of two of them was treated with 2.5 and 5 ppm potassium permanganate, while the third was kept as untreated control. The concentrations of E2, EE2 and BPA assessed by High Performance Liquid Chromatography (HPLC) were 9.150, 16.655 and 0.371 mg/L respectively in ponds water and only 0.125 mg/L bisphenol in tap water. This concentration declined in agriculture water treated by 2.5 ppm, moreover E2 and EE2 were completely eliminated with 5 ppm. As an accurate biomarker for estrogenicity, vitellogenin gene expression was assessed in the livers of all groups. A significant downregulation in farm treated groups was observed compared to control, it was 0.108 and 0.029 fold for 2.5 and 5 ppm, respectively.

Key words: Estrogenic pollution, Potassium permanganate, Vitellogenin, Gene expression, *Oreochromisniloticus*

[Full text-PDF] [RICeST]

Research Paper

Nutritive Value and Dry matter Disappearance of Sudanese Acacia Browse Leaves in Goat Nutrition.

Kamal Al shafei N and Nour A.

World Vet. J. 6(2): 46-52, 2016; pii:S232245681600007-6

ABSTRACT

Browse acacia trees provide feeds of high protein content for livestock in the arid and semiarid regions of Africa. They are important for subsistence livestock production in Sudan. They are a source of high quality protein and minerals for sheep, goats and camels especially in the dry season when the quantity and quality of other feed recourses decline. The nutritive value and digestibility of leaves from five browse trees were analyzed in this study. Browse trees employed in this study are: *Acaciabalbida, Acacia nubica, Acacia sieberiana, Balanites aegyptiaca,* and *Ziziphus-spina- Christi*. Leaves were collected from different areas of the semi arid region of the Sudan. The browse samples were analyzed for their chemical composition, fiber fractions and anti-nutritive components of their leaves, and dry matter disappearance rate. Three fistulated goats were used to determine dry matter disappearance rate (nylon bag technique) at different periods of time. The results showed that the browse species studied have good nutrients contents, especially proteins, and have low and safe levels of anti-nutritional factors, and may therefore form good feed resources for ruminant animal production during dry season. The acacia browse leaves have variable amount of lignin and tannin, which might have contributed to the lower DM digestibility of leaves observed for some species in this present study. However, the results of this study revealed that *A .nubica* may be considered to be an ideal browse acacia tree for this area of Sudan, because of its high protein and energy content, low lignin and tannin, and high DM digestibility. The results of this study should encourage more research and serious efforts on the propagation of high quality trees such as *A. nubica*.

Key words: Acacia tree species, Anti-nutritive factors, Nylon bags technique, Sudan

[Full text-PDF] [RICeST]

Research Paper

Species Variation on Gross Morphology and Gross Morphometry of Accessory Sex Glands in One-Humped Camel Bull (Camelus dromedarius), Uda Ram and Red Sokoto Buck.

Abdullahi Mahmud M, Josephat O, Sani Abdullahi Sh, U Muhammadu Aminu, Abdurrahman B, Hena Akawu S, Abubakar D and Shehu S.

World's Vet. J. 6(2): 53-58, 2016; pii:S232245681600008-6

ABSTRACT

Species variation on gross morphology and gross morphometry of accessory sex glands of One-humped Camel Bull (OCB), Uda Ram (UR) and Red Sokoto Buck (RSB) were carried out. Fifteen reproductive systems were collected; the accessory sex glands were grossly examined, and measured for weight or length. All the three studied animals have ampulla, prostate gland and bulbourethral gland. However, there was no presence of vesicular gland (seminal vesicle) in the OCB. Gross morphologically, the ampulla was most pronounced in the UR and least in the OCB. The two ampullae attached to each other through genital fold connective tissues mostly in the UR followed by OCB and then in the RSB. The broadness of the ampulla was also in that order. The seminal vesicles were markedly lobulated, dark-greyed in color and were the largest of the accessory glands. Though curved in both UR and RSB, seminal vesicles were however more pronounced in the UR in which each pair presented a lateral central depression. The prostate gland consisted of only disseminated or scattered parts extending along the pelvic urethra in the UR and the RSB. In OCB, the prostate gland was the largest accessory sex gland. In OCB, prostate glands were found on the dorsolateral aspect of the pelvic urethra above the ischial arch with a thick interglandular septum between them and almond in shape. Also in the RSB, they were found in the same area as in the OCB, but with a relatively less space. Gross morphometrically, results of the mean ampulla lengths and weights of OCB, UR and RSB showed that the means were significantly different. The results of the mean vesicular gland weights of UR and RSB indicated a significant difference. The results of the mean lengths, weights and diameters of bulbourethral gland showed that the means were significantly different in the three studied species. It was concluded that although results show that the studied animals are different ruminant species they exhibit some similarities and interesting morphological differences in gross morphology and gross morphometry of their accessory sex glands compared to the majority of mammals. The basic morphological characterizations done in this study are important for future studies, such as comparison with other species of ruminants (whether true or pseudo).

Key words: Species variation, Gross morphology, Gross morphometry, Accessory sex glands, Red Sokoto buck, Uda ram, One-humped camel bull.

[Full text-PDF] [RICeST]

Research Paper

Cattle Fattening, Constraints and Marketing System in North Western Ethiopia.

Addisu Mekuria Sh.

World Vet. J. 6(2): 59-65, 2016; pii:S232245681600009-6

ABSTRACT

A study was conducted from January to May, 2015 with the objective to assess cattle fattening and marketing system in north western Ethiopia of north Gondar zone by interviewing 120 cattle fattener households selected purposively. 40% of household source of income were livestock production. Half of the respondents selecting cattles for fattening were red coat color and 80% of them were castrating male animals before the commencement of fattening. Fattening length and age for oxen were 3 months and 7 years old, respectively. From the total of respondents the major sources of feed used for cattle fattening were bean straw (26.67%), nug cake (23.33%), chick pea (16.67%), wheat bran (13.33%), barely straw (10%) and teff straw and hay (3.33%). Decisions on end of cattle fattening period were considering on rate of live weight change (56.67%). Market of fattened animals was during main holidays. The price was highest from February to June, whereas low from September to January. Marketing of beef cattle practiced by bargaining and farmers, cattle traders, whole sales and retailers were involved. The maximum and minimum price of fattened cattle in the dry and wet season was 20000 ETB, 10000 ETB and 13000 ETB, 8000 ETB, respectively. Lack of capital (40%) were the main constraint to begin cattle fattening and other constraints were shortage of feed and water, insufficient land, occurrence of disease and lack of awareness in order of importance 26.67%, 16.67%, 10%, 6.67%, respectively. Therefore, from the present study, it can be conclude that cattle fattening in north western Ethiopia of north Gondar zone is one of the potential strategy to improve the livelihood of the family and had a good potential of market flow.

Key words: Cattle fattening, Constraint, Marketing

[Full text-PDF] [RICeST]

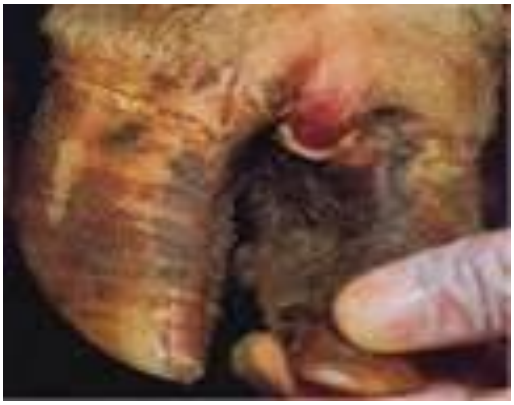
Case Report

Diagnosis and Therapeutic Management of Tetanus in Female Buffalo Calf at Tandojam, Sindh, Pakistan.

Khan A, Abbas Raza SH, Saeed M, Arain MA, Shoaib M, Babazadeh D, Abbasi IHR, Muhammad Zakaria H, Ali Siyal F and Nawaz Soomro R.

World Vet. J. 6(2): 66-69, 2016; pii:S232245681600010-6

ABSTRACT



A female buffalo calf with wound on left leg just below the knee joint suffering from high and persistent fever, anorexia, difficult mastication and urination, stiffness in neck muscle and with some degree of bloat was brought to department of veterinary medicine faculty of animal husbandry and veterinary sciences, Sindh agriculture university, Tandojam, Pakistan, and admitted. The calf was diagnosed to be suffering from tetanus based on clear cut symptoms of high fever, stiff muscles, urine retention and fixed jaws. The Graham's staining of the fresh smear revealed gram+ve rod shape bacteria that appeared like drumsticks. Furthermore, the *Clostridium tetani* was cultured and isolated from the deep necrotic tissue of the wound. The calf was treated with high doses of procaine penicillin, anti-tetanus serum, sedative, meloxicam and intravenous fluid electrolyte therapy (Dextrose 5%). The calf was feed through stomach tube and the urinary catheter was administered to ease out the problem of urine retention. After continues therapeutics management, the calf recovered in two weeks.

Key words: Buffalo calf, *Clostridium tetani*, Diagnosis, Therapeutic management

[Full text-PDF] [RICeST]

Research Paper

Cadmium Bio-Accumulation and the Associated Biomarkers in Edible Frog Species (*Hoplobatrachus Occipitalis*) in Ibadan, Oyo State, Nigeria.

Abosede Olayemi O, Afusat Jagun J, Oluwatobi Felix A, Augustine Adewole A.

World Vet. J. 6(2): 70-79, 2016; pii:S232245681600011-6

ABSTRACT

The spate of natural emissions and anthropogenic activities has comparatively increased cadmium pollution in recent times. This has also increased the attendant hazardous implication on both the aquatic and terrestrial ecosystems. In this study, a total of 50 edible frog species (*Hoplobatrachus occipitalis*) sourced from the Ogunpa river in Ibadan, Oyo state were sampled. Atomic Absorption Spectrophotometry (AAS) was used for the evaluation of the blood, kidney and liver cadmium level. The frogs were grouped into Below Permissible Limit (BPL) and Above Permissible Limit (APL) groups using the FAO/WHO cadmium permissible level of 0.5mg/kg. 86% of the sampled frogs had blood cadmium level above the permissible limit while the liver and kidney cadmium levels exceeded the permissible limits in all the frogs. The highest cadmium level was detected in the liver (3.02±1.23 mg/kg). The erythrocyte parameters were significantly lower in the APL compared to the BPL group while the leucocyte parameters were higher in the APL than the BPL group. The histopathological lesions were consistent with pathological changes associated with renotoxic, hepatotoxic and reproductive features of cadmium toxicity. The study highlights the elevated cadmium levels in the tissue of the frog as a biomarker of exposure while the haematological and histopathological changes served as biomarkers of effect associated with cadmium toxicity in naturally exposed frogs. It also serves to underscore the importance of frogs as important sentinels of environmental cadmium toxicity, creation of public health awareness for cadmium toxicity and the evaluation of cadmium toxicity in the ecosystem.

Key words: Cadmium, Bio-Accumulation, *Hoplobatrachus occipitalis*, Biomarkers, Toxicity

[Full text-PDF] [RICeST]

Research Paper

Isolation and Identification of *Brucella* Species from Dairy Cattle by Biochemical Tests: The First Report from Ethiopia.

Geresu MA, Ameni G, Wubete A, Arenas-Gamboa AM, Mamo Kassa G.

World Vet. J. 6(2): 80-88, 2016; pii:S232245681600012-6

ABSTRACT

Isolation of *Brucella* organism is considered as the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant for control of brucellosis using vaccination. Serological studies revealed that brucellosis is endemic in bovines in Ethiopia. Even though seroprevalence of brucellosis is established in different species of animals, so far there was no successful attempt to isolate and identify *Brucella* spp. in dairy cattle at farm level in the country. Therefore, the endeavor of the present study was to isolate *Brucella* spp. from seropositive cattle with a history of abortion. A total of 570 dairy cattle from 35 herds were screened serologically by Rose Bengal plate test based on the history of abortion in the farm. Among the tested samples 13 (2.28%) were found positive by Rose Bengal plate

test screening while 33 samples were found sero negative upon serological screening test but were collected from the cattle with history of recent abortion. Forty six clinical samples were cultured which were both from *Brucella* seropositive and seronegative (dairy cattle with history of abortion) upon Rose Bengal plate test screening. Three (6.52%) samples were *Brucella* culture positive and further characterization of all the three isolates based on biochemical tests result confirmed that the pathogen was *Brucella abortus*. *Brucella abortus* was isolated from placental cotyledon 1/9 (11.1%) and vaginal swab 2/23 (8.69%) while no isolate was obtained from milk and fetal abomasal contents (abomasal aspirate) of aborted fetus. Our finding revealed the occurrence of *B. abortus* in dairy cattle of Ethiopia through isolation of the organism for the first time from seropositive dairy cattle with a history of abortion. The organisms were isolated from placental cotyledon (one isolate) and vaginal swab (two isolates) while no isolate was obtained from milk and fetal abomasal contents (abomasal aspirate) of the aborted fetus. Hence, the bacteriological isolation and identification of *Brucella abortus* from dairy cattle indicates the importance of brucellosis in dairy cattle industry of the area and potential public health implication for human population in the study areas.

Key words: Isolation, Dairy cattle, *Brucella abortus*, Biochemical test, Ethiopia

[Full text-PDF] [RICeST]

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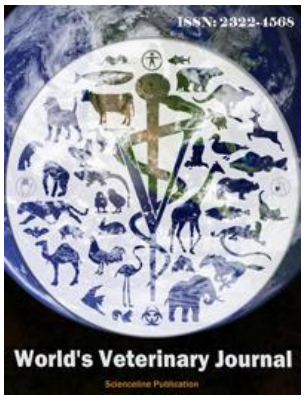
World's Veterinary Journal



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Potassium Permanganate Alleviates the Potential Effect of Estrogenic Pollutants on Vitellogenin Gene Expression in Male *Oreochromis niloticus*

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ABSTRACT

This study aimed to determine the concentration of some estrogens like 17β-estradiol (E_2), 17α-ethynylestradiol (EE_2) and bisphenol A (BPA) in agriculture drainage water that used in Egyptian fish farms and to evaluate the oxidative degradation effect of potassium permanganate on these estrogenic pollutants. In addition to assess vitellogenin gene expression on mature male *Oreochromis niloticus* as a biomarker for estrogenisity. Three groups of males were allocated in three farm ponds filled with agriculture drainage water used in Egyptian fish farms. Water of two of them was treated with potassium permanganate in a concentration of 2.5 and 5 ppm while that of the third pond was kept as untreated control. Other three groups were allocated in three laboratory glass aquariums filled with tap water. Water of two of them was treated with 2.5 and 5 ppm potassium permanganate, while the third was kept as untreated control. The concentrations of E_2, EE_2 and BPA assessed by High Performance Liquid Chromatography (HPLC) were 9.150, 16.655 and 0.371 mg/L respectively in ponds water and only 0.125 mg/L bisphenol in tap water. This concentration declined in agriculture water treated by 2.5 ppm, moreover E_2 and EE_2 were completely eliminated with 5 ppm. As an accurate biomarker for estrogenicity, vitellogenin gene expression was assessed in the livers of all groups. A significant downregulation in farm treated groups was observed compared to control, it was 0.108 and 0.029 fold for 2.5 and 5 ppm, respectively.

Key words: Estrogenic pollution, Potassium permanganate, Vitellogenin, Gene expression, *Oreochromisniloticus*

ORGINAL ARTICLE
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INTRODUCTION

In spite of industrial sophistication and human made chemicals which have made our lives more convenient, nevertheless at the same times they may also cause unforeseen adverse effects on both humans, wildlife (Nakamura et al., 2015) fish (Hallgren et al., 2014) and their reproduction (Barucca et al., 2006). Many Endocrine Disrupting Chemical substances (EDCs) may behave as estrogen mimics including pesticides, insecticides, surfactants, plasticizers (Denslow et al., 2001) and fertilizers (Verderame et al., 2016). Estrogens disrupt endocrine and other vital systems when present in the aqueous environment and increase the risk of cancer, even at nanograms (Benhamou and Sarasin, 2002). Exposure of mammals to Bisphenol A (BPA), one of estrogenic EDCs, may result in decreased steroidogenesis, inhibition of testis growth with low semen quality, disturbance in follicle growth and oocyte meiotic abnormalities (Zhang et al., 2016). BPA also showed adverse effects on the immune system and lipid metabolism (Rogers et al., 2013). Multiple adverse effects due to exposure to estrogens have been reported such as fish feminization, delayed sexual maturation, reduced gonadal growth and altered steroidogenic capacity (Rodas-Ortíz et al., 2008). The most potent chemicals potentially causing adverse effects on fish species are estrogens in human waste (Nakamura et al., 2015). Egyptian Nile river receives many pollutants like effluents of fertilizer and pesticides factories (Osman et al., 2015). Moreover, it was estimated that the Nile river receives more than three million cubic meters daily of untreated or partially treated domestic wastes and municipal sewage (El Gammal and El Shazely, 2008).

In Egypt the most intensive fish farms receive water from agricultural drains to which industrial outfalls and sewage are discharged. Sewage effluent contains many thousands of chemicals, only some of which have been identified. Some, if not many, of the unidentified compounds will possess estrogenic activity. Fish living in waters contaminated with sewage effluent are therefore probably exposed to a mixture of estrogenic chemicals (Harries et al., 1997) which are absorbed and bioaccumulated in sufficient concentrations to induce adverse physiological responses in fish (Sumpter and Jobling, 1995) that could negatively affect their reproduction (Pereira et al., 2015).

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Both females and males have Vitellogenin (VTG) gene but the lack of estrogens in the males prevents the expression of the protein under normal conditions (Sundararaj et al., 1982). The ability of many EDCs to mimic the estrogen can lead to unscheduled hepatic synthesis of VTG in male fish (Arukwe and Goksøyr, 2003). As male fish possess the hepatocyte Estrogen Receptor (ER), it can synthesize VTG when exposed to natural estradiol or other estrogen mimics (Virk et al., 2014). The induction of VTG in male fish is an indicator of exposure to estrogen or estrogen mimics. So, VTG induction has been suggested to be a useful tool for the screening of endocrine disruption and potentially concerning estrogenicity (Gröner et al., 2015).

Natural and synthetic estrogens are not completely broken down by current wastewater treatment processes and achieve minimal levels of removal (Ternes et al., 1999). Therefore, effective treatment approaches are very essential for the decomposition of the estrogenic EDCs from wastewaters. Chemical oxidation can convert hazardous pollutants to nonhazardous or less toxic compounds, and may be a good choice to eliminate EDCs in wastewaters (Shao et al., 2010). The common chemical oxidants applied during water treatment include ozone, chlorine and permanganate but both ozone and chlorine can react with bromide to form brominated by-products which have potentially worse health effects (Guan et al., 2010), while permanganate compared to other oxidants is sometimes preferred because of several advantages, it has a relatively low cost, ease of handling, comparative stability and effectiveness over a wide pH range (Zhang et al., 2013) and a wide range of temperature (Shao et al., 2010). Permanganate oxidation is an effective method for clearing waters containing phenolic EDCs. Some researchers reported the kinetics and mechanism of EDCs degradation using permanganate in aqueous solution like BPA (Jiang et al., 2010) and progesterone (Fayad et al., 2013). The study aims to assess the level of estrogenic contaminants specially17β-estradiol (E_2), 17α-ethynylestradiol (EE_2) and Bisphenol A (BPA) levels in water of agricultural drains that are used in intensive fish farms in Egypt and tap water also to evaluate the efficiency of potassium permanganate in degradation of these substances and using VTG gene expression in male *O. niloticus* as a monitor for change in estrogenicity.

MATERIAL AND METHODS

Fish and rearing conditions

All procedures and investigations were reviewed and approved by the animal ethical committee of faculty of veterinary medicine, Kafrelsheikh University and were performed in accordance with the guiding principles for the care and use of laboratory animals. Seventy two sexually mature male *O. niloticus* were randomly selected. Thirty six were kept in three fish farm hatchery in outdoor concrete ponds for12 days with natural photoperiod (14 hours light and 10 hours dark) in one of Kafr el-Sheikh governorate fish farms during May, the reproductive season. These ponds used agricultural drainage water, their temperature was 25±2°C and the pH value was in the range of 7.5 to 8.5. Fish were fed twice daily on commercial fish food (28% crude protein). The other fish were kept in three glass aquariums (100× 30 ×50 cm)which were filled with aerated and de-chlorinated tap water (Place the water outside in a sunny location, the chlorine will escape the water in the form of a gas) under laboratory conditions, the same like above.

Experimental design

Three groups of male *O. niloticus* were allocated in three farm ponds. Potassium permanganate was added to water of two ponds, the 1st and 2nd had a concentration of 2.5 and 5 ppm respectively according to Xiao-Yan et al. (2015) while the 3rd was kept as untreated control. At the same time three groups were allocated in three laboratory glass aquariums. Water of two of them was treated with potassium permanganate in a concentration of 2.5 and 5ppm while that of the 3rd was kept as untreated control. E_2, EE_2 and BPA were determined in both treated and untreated water using HPLC. VTG gene expression was assessed in livers of males in all groups using real time PCR.

Samples

Water samples were collected for determination of E_2, EE_2 and BPA from both the three farm ponds and the three laboratory aquariums. Liver samples from randomly collected males were obtained, preserved in RNA later solution (preserve RNA in tissue from damage) then stored at -20·Cuntil RNA extraction.

Chemicals

$E_2$with a purity ≥ 98%, EE_2 with a purity ≥ 98%and BPA with a purity = 97% were purchased from Sigma-Aldrich and used without further purification. Acetonitrile and methanol (HPLC grade) were also obtained from Sigma-Aldrich. Potassium permanganate, organic solvents and the other chemicals used were analytical grade.

Analytical method

High performance liquid chromatography (HPLC) was used. The separation conditions were carried out according to Naimi and Bellakhal (2012) with some modifications to determine the concentrations of E_2, EE_2 and BPA in water

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samples. The separations were carried out by HPLC system (YL9300 series), which consisted of a quaternary pump, manual sample injector with a 20 µL loop and a UV/VIS detector. Analytical separations were achieved on by reversed phase C-18 (Agilent, 5µm; 4.6 mm × 250 mm) column, column temperature was 40°C and UV detection was performed at 230 nm. The mobile phase was a mixture of acetonitrile, methanol and acetic acid 0.1% (55:5:40 (v/v)), with constant flow rate 1 mL/min. Individual standard solutions of E_2, EE_2 and BPA were prepared in acetonitrile. The concentrations of the analytes were calculated by measuring the peak area for each compound and comparing it with those obtained from a standard solution.

RNA extraction and real time PCR

Total RNA was extracted using RNeasy mini kit (QIAGEN) according to manufactures protocol. All primers of real time PCR were synthesized by Bio Basic Canada Inc. oligonucleotide primers used in SYBR Green real time PCR (Gröneret al., 2015) were illustrated in table 1. Preparation of PCR Master Mix was carried out according to Quanti Tect SYBR green PCR kit (QIAGEN). Cycling conditions for Elongation Factor 1α (EF-1α) and VTG gene were illustrated in table 2. Real time PCR data were analyzed using MxPro software (Stratagene) version 4.10 by means of the comparative Cycle Threshold method ($\Delta\Delta C_T$), C_T values are equal to the number of cycles required to reach fluorescence above the threshold level (Pfaffl, 2001). EF-1α was used as an internal standard by performing each PCR of the target gene and EF1-α from the same samples. Expressions of target gene were normalized to the corresponding level of EF1-α mRNA.

Table 1. Primer sequences position, product lengths, annealing temperature (Ta) and GenBank accession numbers of primers used for gene expression quantification of reference gene elongation factor 1α (EF-1α) and VTG gene of *O. niloticus*

Target gene	Primer sequences (5'-3')	Position	Product lengths (bp)	Ta (°C)	GenBankaccession numbers
EF-1α	Forward GCTTCAACGCTCAGGTCATC	1050	86	62	AB075952.
	Reverse TGTGGGCAGTGTGGCAATC	1136			
VTG	Forward CTTTCCATCCAGCCACCAAG	71	160	60	FJ709597.1
	Reverse CTGCAGGAGGTTGATGATGC	231			

Table 2. Cycling conditions for EF-1α and VTG SYBR green real time PCR

Stage	Temperature	Time	Cycles
Reverse transcription	50° C	30 min	1
PCR initial activation step	94° C	10 min	1
Amplification a) denaturation	94° C	30 sec	45
b) Annealing	60° C for VTG & 62° C for EF1α	30 sec	
c) Extension	72° C	45 sec	
Dissociation curve a) Secondary denaturation	95° C	1 min	1
b) Annealing	60° C for VTG & 62° C for EF1α	1 min	
c) Final denaturation	95° C	30 sec	

Data analysis

Statistical significances among the groups were performed using GraphPad Prism 6 software. PCR data are presented as mean ± SE, group differences of gene expression levels were determined by one-way ANOVA. For statistical significance between groups, one way ANOVA was followed by the unpaired t-test, the significance level was set to $P \leq 0.0002$ in farm groups and $P \leq 0.005$ for laboratory groups.

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Journal homepage: www.wvj.science-line.com

RESULTS

Concentration of E_2, EE_2 and BPA and effect of potassium permanganate on their degradation

HPLC analysis for fish farm water revealed that the concentration of E_2, EE_2 and BPA were 9.150, 16.655 and 0.371 mg/L respectively. While in tap water, analysis showed absence of E_2 and EE_2 while 0.125mg/L BPA was detected. Treatment of agriculture water with potassium permanganate at a concentration of 2.5 ppm reduced the concentrations to be 8.457, 4.776 and 0.094 mg/L respectively. The increase of potassium permanganate concentration to 5ppm resulted in complete elimination of E_2 and EE_2 and slight decrease in BPA concentration to 0.090 mg/L (Figure 1).Treatment of tap water with 2.5 ppm potassium permanganate reduced the concentration of BPA to be 0.112 mg/L. Increase of potassium permanganate concentration to 5ppm resulted in the complete elimination of BPA (Figure 2).

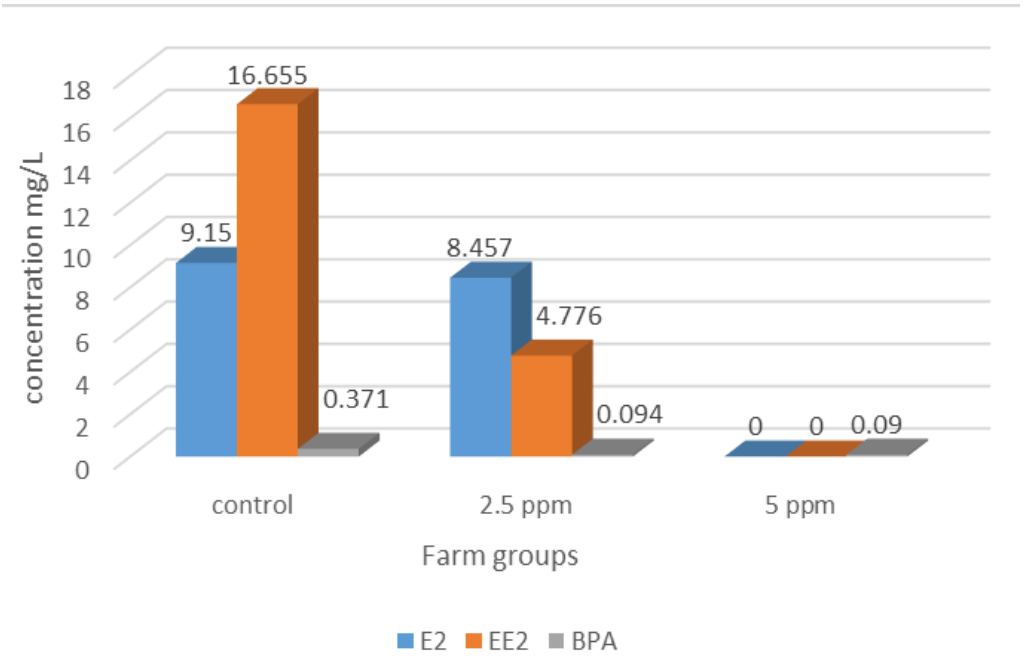

Figure1. Effect of 2.5 and 5 ppm potassium permanganate in reduction of E_2, EE_2 and BPA concentrations in farm water

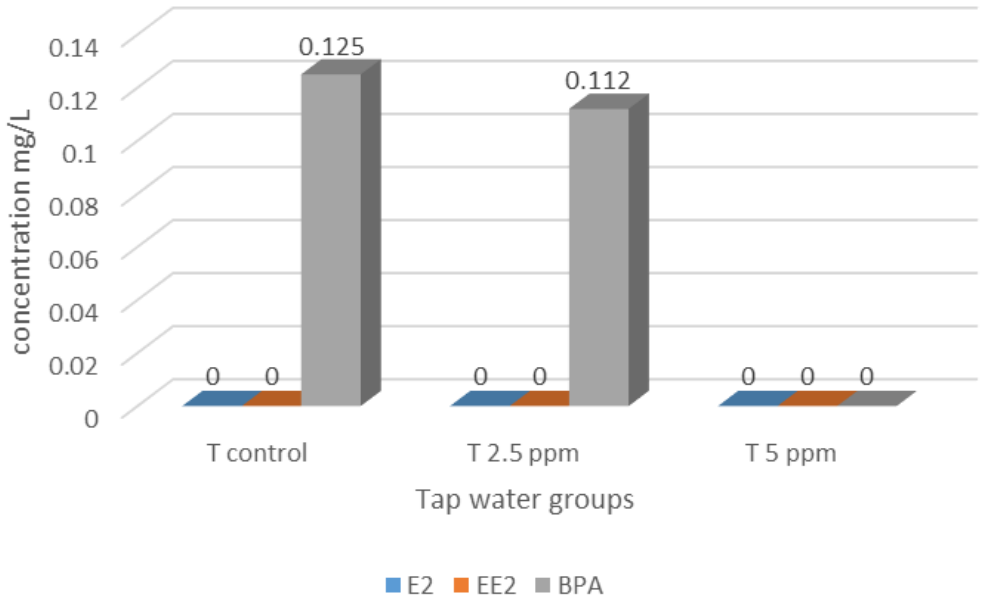

Figure 2. Potassium permanganate effect in reduction of bisphenol A (BPA)to be 0.112 mg/L after treatment of tap water by 2.5 ppm while increase the concentration to 5 ppm resulted in complete elimination of BPA

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VTG gene expression

In comparison with the control farm group, treatment of water by 2.5and 5ppm potassium permanganate caused significant down regulation in expression of VTG gene in *O. niloticus* livers for the two concentrations. Treatment by 2.5ppm showed a decrease of 0.108 fold while 5ppm leads to a 0.029 fold decrease(Figure 3).While in tap water, the group treated with 2.5ppm potassium permanganate showed slight change to be 1.103 fold in comparison with control while in 5ppm group the significant decline was 0.507fold (Figure 4).

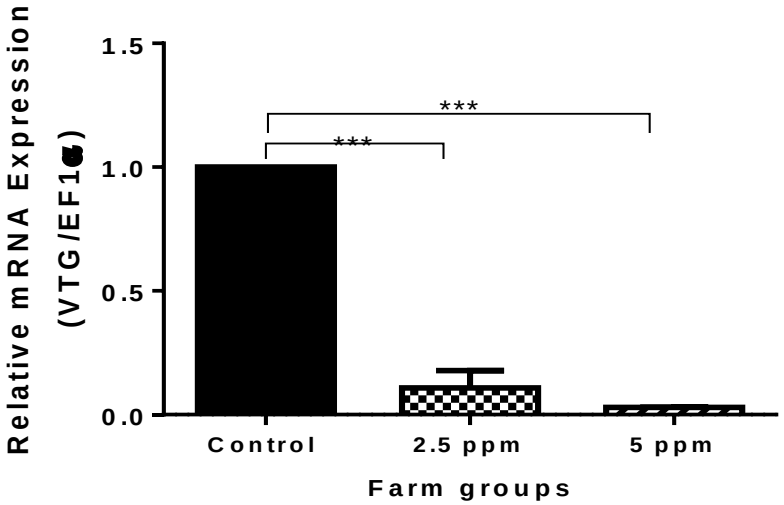

Figure 3.The fold changes (means ±SE) in mRNA expression of Vitellogenin(VTG) gene relative to Elongation Factor 1-α (EF1-α) gene in the farm groups. Asterisks indicate statistically significant differences to control. P ≤0.0002 (t-test for unpaired values).

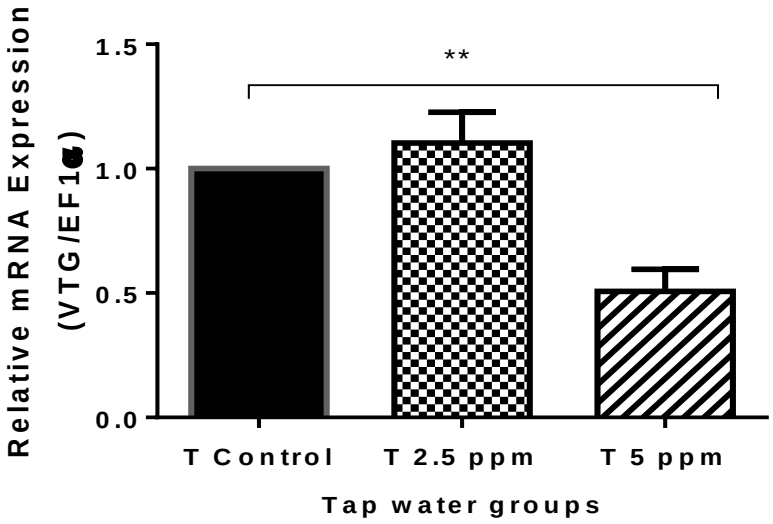

Figure4.The fold changes (means ±SE) in mRNA expression of Vitellogenin (VTG) gene relative to Elongation Factor 1-α (EF1-α) gene in tap water groups. Asterisks indicate statistically significant differences to control. P ≤0.005 (t-test for unpaired values).

DISCUSSION

The usage of agriculture drain water carrying many EDCs, in aquaculture may cause environmental hazards on the fish populations in these farms. Scientific research in this area must focus on how to mitigate or eliminate these hazards. EDCs including natural steroid and synthetic estrogens have been verified as the common form of estrogenic activity in water (Kolpin et al., 2002 and Snyder et al., 2001). The actual concentrations of EDCs in environmental samples varies from ng/L to µg/L (Shao et al., 2010). In the present study, the concentration of E_2, EE_2 and BPA in fish farm water

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samples were 9.150, 16.655 and 0.371 mg/L respectively and the concentration of BPA in tap water was 0.125 mg/L. The concentrations are high in farm water because it receives numerous nonpoint and point sources of pollution like untreated industrial, municipal and agricultural wastes in addition to sewage which contain many chemicals that possess estrogenic activity (Flammarion et al., 2000).

Chemical oxidation may be a good choice to eliminate EDCs in natural waters. Permanganate has been described as an inexpensive, easy and effective oxidant for degradation of EDCs (Abe et al., 2003). Permanganate oxidation has already been used in drinking water treatment processes (Shao et al., 2010). Beside, Potassium permanganate has been used for many years in aquaculture as an effective oxidizer for control of many bacterial, parasitic and fungal agents, eliminating the need for antibiotic therapy (Francis-Floyd and Klinger, 2009). Permanganate may have a high selectivity for EDCs oxidation in water (Guan et al., 2010). Permanganate mainly reacts with double bonds by donating oxygen, but it can also accept electrons or hydrogen atoms (Stewart, 1964). The mechanism for the oxidation of phenolic EDCs by permanganate was a single electron transfer and aromatic ring cleavage (Guan et al., 2010). In the farm experiment, treatment of water with potassium permanganate at a concentration of 2.5 ppm reduced the concentration of E_2, EE_2 and BPA from 9.150, 16.655 and 0.371 mg/L to 8.457, 4.776 and 0.094 mg/L respectively. Increase of potassium permanganate concentration to 5ppm resulted in the complete elimination of E_2 and EE_2. In other wards the efficiency of degradation of these EDCs is correlated with permanganate concentration. This is in agreement with results of Xiao-Yan et al. (2015) who stated that E_2 concentration decreased with increasing permanganate dosage and the removal efficiency of E_2 was greater than 99.6% when the reaction reached 30 min with 5 mgL^{-1} permanganate. Xiao-Yan et al. (2015) also compared between the E_2removal efficiency of ultrasound/potassium permanganate, potassium permanganate and ultrasound water treatment processes, the results showed that the ultrasound/potassium permanganate process was the most effective process among the three, followed by potassium permanganate and then ultrasound. It can be concluded that in the process of ultrasound/potassium permanganate, potassium permanganate played a promotional action in the degradation of E_2.The results of our study showed that addition of permanganate alone was as efficient as to bring the concentration of estrogens in agriculture drained water to nearly the same concentration in the very expensive tap water.

Biomarkers have the ability to improve accuracy, reliability and scientific basis for the quantitative assessment of environmental health risks (Arukwe and Goksøyr, 2003). VTG in male fish is an ideal biomarker to study the estrogenicity of EDCs on fish (Gröner et al., 2015; Virket al., 2014; Yamaguchi et al., 2015). Measurement of VTG mRNA expression in male fish liver is a rapid accurate method for detecting changes in VTG in fish exposed to estrogens and for monitoring estrogenic exposure (Barucca et al., 2006). Levels of VTG mRNA increase after exposure to estrogenic pollutants (Scholz et al., 2004). VTG mRNA in fish continuously exposed to estrogens is upregulated in a dose dependent manner also VTG mRNA transcription is induced immediately and its half life is short as it is quickly degraded in the absence of estrogen (Bowman et al., 2000). The results of our study showed adown regulation of the estrogenic biomarker VTG gene expression in the livers of male *O. niloticus* that reared in water treated with potassium permanganate, revealed elimination of estrogenic potential via oxidation of E_2, EE_2 and BPA by potassium permanganate and decrease their adverse effect on fish.

CONCLUSION

E_2, EE_2 and BPA are present with a relatively high concentration in agriculture drainage water which used in fish farms in Kafr El-Sheikh governorate. VTG gene expression in male *O. niloticus* may be considered a valuable biomarker for exposure to environmental estrogens. Contamination of fish farm water with estrogenic material can activate VTG gene expression in male fish which is normally inactive. The changes in mRNA level can be used as fingerprints to characterize an estrogenic exposure. Adding potassium permanganate in a dose 2.5 and 5 ppm can induce oxidative degradation of these estrogens and this lead to downregulation VTG expression in male fish.

Competing interests

The authors have no competing interests to declare.

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Nutritive Value and Dry Matter Disappearance of Sudanese Acacia Browse Leaves in Goat Nutrition

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ABSTRACT

Browse acacia trees provide feeds of high protein content for livestock in the arid and semiarid regions of Africa. They are important for subsistence livestock production in Sudan. They are a source of high quality protein and minerals for sheep, goats and camels especially in the dry season when the quantity and quality of other feed recourses decline. The nutritive value and digestibility of leaves from five browse trees were analyzed in this study. Browse trees employed in this study are: *Acaciabalbida, Acacia nubica, Acacia sieberiana, Balanites aegyptiaca,* and *Ziziphus-spina- Christi*. Leaves were collected from different areas of the semi arid region of the Sudan. The browse samples were analyzed for their chemical composition, fiber fractions and anti-nutritive components of their leaves, and dry matter disappearance rate. Three fistulated goats were used to determine dry matter disappearance rate (nylon bag technique) at different periods of time. The results showed that the browse species studied have good nutrients contents, especially proteins, and have low and safe levels of anti-nutritional factors, and may therefore form good feed resources for ruminant animal production during dry season. The acacia browse leaves have variable amount of lignin and tannin, which might have contributed to the lower DM digestibility of leaves observed for some species in this present study. However, the results of this study revealed that *A .nubica* may be considered to be an ideal browse acacia tree for this area of Sudan, because of its high protein and energy content, low lignin and tannin, and high DM digestibility. The results of this study should encourage more research and serious efforts on the propagation of high quality trees such as *A. nubica*.

Key words: Acacia tree species, Anti-nutritive factors, Nylon bags technique, Sudan

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INTRODUCTION

Browse shrubs and trees in the tropics have higher protein than grasses (Schoenian, 2009). In Africa, browse trees and shrubs not only provide feed for animals but are often used for other purposes such as source of wood, fruits, or medicine for the local communities and households. The browse shrubs and trees provide an important source of feed for livestock arid and semiarid zones of tropical Africa (Von Kaufmann, 1986). Ruminants in arid and semiarid areas of Africa suffer from shortages of feeds and their poor quality, especially during the dry season (Shelton, 2004). Browse trees and shrubs remain as an important source of better quality feed for sheep, goats, and camels, and their nutrients can provide protein and mineral supplements to improve the productivity of livestock that feed on low quality forages and crop residues (Aganga and Tshwenyane, 2003). As the dry season progresses, feed in dry lands become inadequate in quantity and quality and this leads livestock, such as camels and goats, to depend more on perennial vegetation and browse trees which provide leaves, edible branches and fruits (Abdelgabar, 1986). Browse trees and shrubs can be credited for supporting sustainable subsistence livestock production in arid and semi-arid zones of Africa; since the feeds they provide have high crude protein (CP) and mineral contents. The edible parts (leaves and fruits) of the majority of browse trees and shrubs have more than 10% CP, even in the dry season, when CP% progressively decreases (Backlund and Belskog, 1991). Poor quality feed tends to take longer to digest than high quality feeds, and digestibility of nutrients tend to decrease with increasing the amount of roughage, especially poor quality one (Chessmore, 1979). Lignin is an important anti-nutritional factor that decreases the digestibility of nutrients in fodder crops (Minson, 1990). Lignin is also a limiting factor in the digestion of legumes, but its affect is less pronounced as that of cereal forages (Rittner and Reed, 1992).The objective of this study was to determine the chemical composition, anti-nutritional constituents of leaves from five browse acacia trees (*Acacia albida, Acacia nubica, Acacia sieberiana, Balanites aegyptiaca,* and *Ziziphus spina- Christi*), and dry matter disappearance, using nylon bags technique, after the digestion in the goat rumen for different periods of time.

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MATERIAL AND METHODS

Experimental animals and duration of experiment

Three female goats were used in this experiment, those goats from Nilotic species, all goats were in the same weight (~20 Kg), and age (~2 years), and all are healthy. The animals were housed separately under hygienic conditions in a cleanroom with adequate. Institutional procedure for handling experimental animals was followed. Light and good ventilation .All animals were fed with Berseem (*Medigaco sativa*). The rumen fistula was made by a surgical operation in the left side of the animal, so the diameter of passageway connecting the goat's rumen with the skin is large enough to administrate the samples in the nylon bags. Animals were allowed two weeks to heal and acclimatize before the start of the experiment. The experiment took another two weeks.

Collection of feed material

In the present study, leaves were obtained from five acacia trees, growing in different parts of semiarid areas of Sudan. Samples were processed and analyzed for their chemical composition and nutritive value. The samples were carefully cleaned and freed from stones, dirt, or grit; they were then numbered and carefully stored in polythene bags. Their local names, botanical names of the five browse trees used in the study are presented in Table 1.

Table 1. Local and Botanical Names of Acacia trees

Local name	Botanical name
Haraz	*Acacia albida*
Laot	*Acacia nubica*
Kog	*Acacia sieberiana*
Higleg	*Balanites aegyptiaca*
Sidir	*Ziziphus spina- christi*

Nylon bag

The size of the bags was large enough relative to sample used so as to ensure that the ruminal fluid can easily enter the bag and mix with sample. The bag is also small enough to be easily withdrawn through the rumen fistula. The mesh size of these bags allows entry of rumen microbes and exit of fermentation gases on one hand. On the other hand, the losses of solid particles from the bags would be minimum.

Analytical methods

Determination of the chemical composition of the browse plants: The determination of Crude Protein (CP), Crude fiber (CF), Ether Extract (EE, %fat), ash and moisture in the browse leaves were carried out according to the method of analysis of the Association of Official Agricultural Chemist, AOAC (1965). Lignin was determined according to the method of Goering and Van Soest (1970). Quantitative estimation of tannin was carried out using the modified vanillin HCL method described by Price et al. (1987). On the other hand, cellulose and Acid Detergent Fiber (ADF) were digested with acetic acid and sulphuric acid, respectively. The methods used are a modification of Crampton and Maynard (1938).The dry matter disappearance rate in the rumen was determined according to the method of the nylon bag technique of Oroskov et al. (1980).

Preparation of the sample for incubation: Prior to their use, the bags were thoroughly washed under tap water, dried to a constant weight at 105 C° in a hot air oven, and weighed. Four grams of the sample were placed in the bag.

Incubation of the bags in the rumen: In this experiment the fistulated goats were used to determine the dry matter disappearance rate of browse leaves using the nylon bags technique. Four gm of the samples were put in the bags which were inserted into the rumen through the fistula. The nylon bags were identified before incubation by the aid of markers. The bags were incubated for 6, 12, 24, 48 and 72 hrs in the rumen. The nylon bag containing of the browse plant samples were administered directly through the fistula to the rumen. Each sample was replicated 3 times in different three animals and at the end of the incubation period the bags were washed under gently falling stream of tap water, cleaned by rubbing between the finger and the thump until rinsing was clear. The washing time average was 10min/bag, and then the bags were dried for 24hrs at 100C° and weighed. The dry matter losses were determined by calculating the mean of the three replications.

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Statistical analysis

Data were subjected to standard methods of statistical analysis that was performed using windows-based Statistical Package for Social Sciences (SPSS) Version 17.0. Descriptive statistic was used to evaluate the minerals disappearance in trees and shrubs.

RESULTS

In this study, three tables and one figure provide the names of the acacia browse trees, chemical composition, and dry matter disappearance rate of leave samples obtained from the five acacia trees used in the study. Table 2 presents the percentages of moisture, protein, ash, ADF %, lignin, cellulose, and tannin in the browse plants. Table 2 shows that the moisture content of browse species ranges from 7.86% for *A. seiberiana* to19.0% for *B. aegyptiaca.* The CP% ranges from 8.75% to 21%. *Z. spina-christi's* leaves have the lowest CP% while *A. nubica* has the highest CP%. *A. seiberiana, A. albida and B. aegyptiaca* have 13%, 14.87%and 15.75% CP, respectively. The highest fat% was found in the leaves of *B. Aegyptiaca* (6.15%) and rest of the trees have 3.2-4.86% fat in their leaves.

The species that has the lowest CP%, *A. seiberiana*, has also the highest CF% (30%). At the mean time, it was observed that the ash content of the browse samples in the present study were 8.39 –15.0 % (Table2) and that *B. aegyptiaca* leaves has the highest ash%.

Regarding the high amount of lignin content observed in this study (13.40- 79.6%, Table2), *A. nubica* has the lowest lignin content while *A. albida* has the highest. The leaves of the other browse trees were in between. On the other hand, *A. seiberiana* leaves have the highest cellulose content while *A. Nubica* has the lowest. Interestingly**,** *Z. spina-christi* has the highest tannin (3.9%) while *A. nubica* and *B. aegyptiaca* to have around 0.30% tannin.

Disappearance rate (DM digestibility)

The present findings showed that different browse plants species have different dry matter disappearance rates when incubated in the rumen of goats (Table 2 and Figure 1). With the exception of *A. albida* and *A. seiberiana*, leaves from acacia browse in the present study have shown higher (more than 50%) DM disappearance rate, and a higher DM digestibility. *A. nubica*, which has the highest DM disappearing rate, has also high protein content, low lignin and low tannin. This shows the influence of chemical composition on DM digestibility.

Table 2. The proximate chemical composition of *Acacia albida, Acacia nubica, Acacia sieberiana, Balanites aegyptiaca,* and *Ziziphus spina- christi*

Botanical Name	Moisture %	CP %	EE %	CF %	Ash %	ADF %	Lignin %	Cellulose %	Tannin %
A. albida	11.21	14.87	4.86	15.93	8.39	52.00	79.60	20.00	1.50
A. nubica	9.60	21.00	3.53	18.83	12.59	70.00	13.40	14.50	0.36
A. seiberianae	7.86	13.125	3.20	30.36	11.74	18.40	24.00	32.30	1.90
B. aegyptiaca	19.00	15.75	6.15	17.33	15.00	94.20	17.00	21.20	0.32
Z. spina-christi	8.20	8.75	3.49	17.80	9.40	44.20	31.40	24.20	3.16

Crude Protein (CP), Crude fiber (CF), Ether Extract (EE, %fat), Acid Detergent Fiber (ADF %)

Table 3. Dry matter disappearance rate (%) of acacia tress leaves in goats

Botanical name	Incubation periods in hours					
	6 hrs	12 hrs	24 hrs	48 hrs	72 hrs	Means ± SD
A. albida	10.83	20.83	24.17	39.17	41.67	27.36 ± 12.94
A. nubica	25.75	38.25	49.25	70.00	84.25	53.50 ± 23.66
A. seiberianae	17.50	19.17	27.50	40.00	41.67	29.17 ± 11.32
B. aegyptiaca	22.50	29.17	47.50	64.17	66.67	46.00 ± 19.97
Z.spina-christi	11.67	14.17	38.00	51.67	63.33	35.77 ± 22.72

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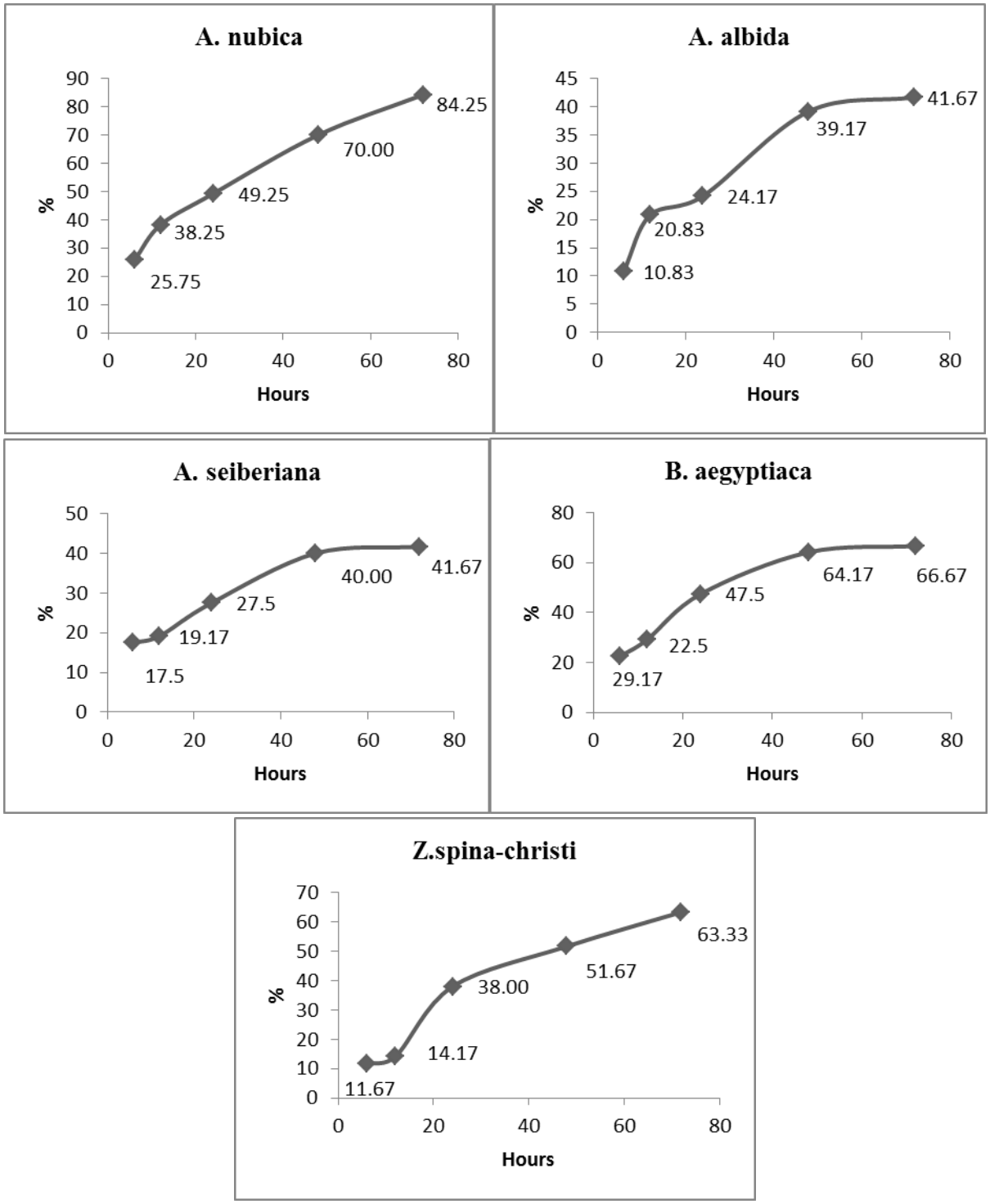

Figure 1. Dry matter disappearance rate for acacia browse leaves over time (6-72 hours)

DISCUSSION

The value of a feed for an animal depends on the feed intake, nutritional content, dry matter digestibility as well as the availability of the nutrients to animal after absorption (Seoane et al., 1981).When feed DM digestibility decreased to less than 50%, the feed did not meet cattle's requirements for nutrients (Leng et al., 1992). Therefore, it is important to analyze the nutrients contents, DM digestibility, and presence of anti nutritional factors in order to determine the worth of a feed for the ruminant animal.

The findings of the present study showed that the moisture content of browse species ranges between 7.86 to –19.0% which is lower than that reported by Le Houeru (1980). Moisture content is affected by rainfall, temperature, and age of the plant. On the other hand, the present findings showed that acacia trees examined in this study had relatively high level of crude protein (8.75 –21.0%) which lies within the range obtained by Walker (1980). Some browse species showed in the present study high level of crude protein, including *A. nubica*, that has21.0 CP% (Table2), which is in agreement with Dougall et al (1964). It seems that the protein content reported in this study would be adequate to meet

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goats requirements, however, not all of the protein will be digested since browse tree forages has been reported to have a true digestibility of proteins of approximately 60-70 % (Wilson, 1977). Lower protein digestibility could be due to the presence of tannin that decreases protein digestion by rumen microbes (Gartner and Hunwood, 1967).When tannin complexes with proteins, it tends to decrease the proteins digestibility (Kumar and Singh 1984). On the other hand, forages that has less than 8% CP can adversely affect growth and function of rumen microbes (Van Soest, 1982). None of the browse acacia species in the present study has CP% of less than 8%.

Browse plants in the present study are high in EE% (Table 2). This is in agreement with Mecha and Adegobla (1980), who reported that high energy content is expected if the edible browse has high content of true fat. Van Soest (1982) contended that the most important factor that makes feed valuable for maintenance of body functions is its metabolizable energy content, especially in dry season because of reduced intake of low quality pasture (Walker, 1957).Wilson and Harrington (1980) reported that when the digestibility of the grass decreases with age, the metabolizable energy levels of the feed declines. In contrast to grass, browse plants seem to be able to provide more than the energy needed for maintenance of livestock (Toutain, 1980). This is not surprising since browse plants have almost twice the energy that is found in the dry grass (Le Houerou, 1980)

The leaves of the acacia browse trees in the present study had CF% hat ranges from 15.93% for *A. albida* to 30.36 % for *A. seiberiana* (Table 2), which is similar to the range reported by Dougall et al. (1964). High protein and low fiber content of acacia browse would provide high nutritive value feeds for browsing sheep and goat, especially during the dry season when the nutritive value of grasses in arid areas of the tropics declines due to decreasing levels of protein and increasing level of crude fiber (Van Soest, 1982). On the other hand, concerning the high level of ADF% observed in this study (18.4- 94.2%, Table 2) which is higher than the values of 27.20- 50.10% reported for twigs. This variation may be due to the differences between ADF% in leaves and twigs (Elginaid, 1997). The high lignin content observed in the present study was higher than the values obtained by Khazaal et al. (1993) who found that the concentrations of fiber and lignin increase as forages advance to maturity. Lignin provides strength to the cell wall as plant grows and growing plant. Because lignin is resistant to microbial degradation in the rumen, it is expected to result in decreased DM digestibility.

The results for the ash% of the leaves of acacia browse trees observed in present study (8.39 –15.0 %, Table2) is slightly higher than the range reported by Dougall et al. (1964), and higher than the range (3.10- 9.70%) reported by Elginaid (1997). On other hand, tannin levels presented in Table 2, range between 0.32% for *B. aegyptiaca*to3.16% for *Z. spina-christi's* leaves which are within the range 0.18- 9.51%, reported by McKey et al. (1978). As mentioned above, of the browse species investigated in the present study, *Z. spina-christi* showed a high level of tannin in their leaves, which is lower than the 5.84% level reported by Reed et al. (1990).Although it is generally accepted that increased tannin in the feed depresses DM digestibility and availability of nutrients for the ruminant animal, Barry and Manley (1984) suggested that in ruminants, directly condensed tannins (2-3%) have been shown to impart beneficial effects because they reduce the wasteful protein degradation in the rumen by the formation of protein-tannins complex.

Browse leaves digestibility

Wilson and Harrington (1980) argued that lignin content decreases digestibility of DM of browse plants, and that the decreased DM digestibility could be due to the fact that lignin may prevent the degradation of the browse feed leaves cell wall (Richard, 1976). Other factors may have contributed to the low DM digestibility, such as presence of some phenolic compounds (Danny, 1982), and the impact of high tropical temperature on the leaves' chemical composition (Van Soet, 1982). Since browse acacia trees investigated in the present study were grown in semi tropical areas, where the temperature is so high, it is expected that as the leaves mature, the cell wall composition changes and that may have resulted in decreased feed intake and digestibility (Figroid et al., 1972). Further, the size of feed the particles in the present study lies in the range of 0.6 and 1.0 mm, and are incubated in the nylon bags. This may have resulted, in the absence of mastication, addition of saliva, and ruminal mixing in decreasing particles degradation and digestibility as suggested by Weakley et al. (1983).

The present findings showed that different browse plants species have different dry matter disappearance rate when incubated in the rumen of goat. This may be due to the variation of the chemical composition and the nutritive values from one browse plant species to another (Le Houerou, 1980). And it also may be due to the effect of the animal and days of the incubation as Meherz and Oroskov (1977) suggested that variation in situ DM disappearance rate of was largely influenced by the animal factor than by variation among days. Figroid et al. (1972) observed differences in DM disappearance rates of barley and sorghum. It, therefore, could be concluded that lignin and tannin, and their levels in different plant species are important factors that significantly depress degradation of cell wall (Hartly et al., 1989). Another factor that might have contributed to decreased DM digestibility is interactions between the bag surface and fibrous mat in the rumen, and that would affect the disappearance rates of fiber from the bags (Weakely et al., 1983).

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CONCLUSION

Acacia browse leaves investigated in this study have, with the exception of one species, high protein content, which provides good nutrition for sheep, goats and camels especially during the dry season. Feeds in the arid and semi arid areas of the tropics, including Sudan, decrease in quality and quantity during the dry season. Protein nutrition is crucial for maintenance and growth. The acacia browse leaves have variable amount of lignin and tannin, which might have contributed to the lower DM digestibility of leaves observed for some species in this study. However, the results of this study revealed that *A. nubica* may be considered to be an ideal browse acacia tree for this area in Sudan, because of its high protein and energy content, low lignin and tannin, and high DM digestibility. The results of this study should encourage more research and serious efforts on the propagation of high quality trees such as *A. nubica*.

Competing Interest

The authors declare that there are not significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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Species Variation on Gross Morphology and Gross Morphometry of Accessory Sex Glands in One-Humped Camel Bull (*Camelus dromedarius*), Uda Ram and Red Sokoto Buck

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ABSTRACT

Species variation on gross morphology and gross morphometry of accessory sex glands of One-humped Camel Bull (OCB), Uda Ram (UR) and Red Sokoto Buck (RSB) were carried out. Fifteen reproductive systems were collected; the accessory sex glands were grossly examined, and measured for weight or length. All the three studied animals have ampulla, prostate gland and bulbourethral gland. However, there was no presence of vesicular gland (seminal vesicle) in the OCB. Gross morphologically, the ampulla was most pronounced in the UR and least in the OCB. The two ampullae attached to each other through genital fold connective tissues mostly in the UR followed by OCB and then in the RSB. The broadness of the ampulla was also in that order. The seminal vesicles were markedly lobulated, dark-greyed in color and were the largest of the accessory glands. Though curved in both UR and RSB, seminal vesicles were however more pronounced in the UR in which each pair presented a lateral central depression. The prostate gland consisted of only disseminated or scattered parts extending along the pelvic urethra in the UR and the RSB. In OCB, the prostate gland was the largest accessory sex gland. In OCB, prostate glands were found on the dorsolateral aspect of the pelvic urethra above the ischial arch with a thick interglandular septum between them and almond in shape. Also in the RSB, they were found in the same area as in the OCB, but with a relatively less space. Gross morphometrically, results of the mean ampulla lengths and weights of OCB, UR and RSB showed that the means were significantly different. The results of the mean vesicular gland weights of UR and RSB indicated a significant difference. The results of the mean lengths, weights and diameters of bulbourethral gland showed that the means were significantly different in the three studied species. It was concluded that although results show that the studied animals are different ruminant species they exhibit some similarities and interesting morphological differences in gross morphology and gross morphometry of their accessory sex glands compared to the majority of mammals. The basic morphological characterizations done in this study are important for future studies, such as comparison with other species of ruminants (whether true or pseudo).

Key words: Species variation, Gross morphology, Gross morphometry, Accessory sex glands, Red Sokoto buck, Uda ram, One-humped camel bull.

CASE REPORT
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INTRODUCTION

The accessory sex glands; prostate, vesicular, ampullae of vas deferens and bulbourethral glands play an important role in the reproductive process (Chughtai et al., 2005). In dromedaries, the accessory sex glands are: the ampullae, the prostate and the bulbo–urethral (Cowper's) glands while the most important feature, is the absence of seminal vesicles (Mobarak et al., 1976). In sheep and goats their accessory sex glands are the ampullae, vesicular glands, prostate gland and the bulbourethral glands which open and empty their secretion into the urethral passage (Khalaf and Merhish, 2010). The accessory glands greatly contribute to the fluid volume of semen. Their secretions are solution of buffers, nutrients and other substances needed to assure optimum motility and fertility of semen (Hafez, 1974; MC Donald, 1980; Bone, 1988). The secretions of the accessory genital glands constitute 60-90% of total volume of semen (Dukes, 2005). The morphology of these glands varies widely among different mammalian species (Thomson and Marker, 2006).

Many researchers have worked on the individual morphology of the accessory sex glands in mammals including one-humped camel (Aliet al., 1978), sheep and goat (Kundu, 1980; Nissar and Suri, 2012; Gofur, 2015). The enlarged end of the vas deferens near the urethra is the ampullae (Khalaf and Merhish, 2010). In sheep and goat, the ampullae is 6-8 cm in long and 4-8 mm in diameter (Getty, 1975) while Ali et al. (1978) reported an average of 18 cm in one-

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humped camel. The vesicular glands are paired, large, externally smooth, hollow and knobby (Dyce et al., 2010). The prostate gland is unpaired gland about the size of chestnut visible on the outside of the urethra just posterior to the excretory ducts of the vesicular gland (Dyce et al., 2010). Grossly, two portions of the prostate gland maybe distinguished: the compact of external portion (corpus prostate) and the disseminate or internal portion (el-Wishy et al., 1972 and Dyce et al., 2010). The body of the prostate in one-humped camel is oval or circular in shape while the disseminate part of the prostate surrounds the pelvic urethra, lies caudally to the body and extend to its end (el-Wishy et al., 1972).

In sheep and goat, only disseminated part of prostate gland surrounds the pelvic urethra which extends caudally along the pelvic urethra (Bearden and Funguay, 2000). The paired bulbourethral gland consists of right and left club-shaped independent lobes, which lies on the dorsal surface of the caudal part of pelvic urethra and closely related to the bulb of penis (Shively, 1982). Since there is paucity of information on the comparative morphology of accessory sex glands in these in One-humped Camel Bull (OCB), Uda Ram (UR) and Red Sokoto Buck (RSB), this research work was carried out with the aim of filling the lacuna.

MATERIALS AND METHODS

Fifteen accessory sex glands of healthy adult OCB, UR and RSB (five samples per species) were collected from Sokoto metropolitan abattoir. Sokoto metropolis is located on latitudes 10° N and 14° 50′ N and longitudes 7° E, east of the equator, in the extreme northwest of Nigeria (NPC, 2006). Following the collection, they were transported to veterinary anatomy laboratory, Usmanu Danfodiyo University, Nigeria, where gross features of the accessory sex glands were examined and recorded. They were then dissected out for measurements. The weights (g) and lengths (cm) were measured using a weighing balance (Shimadzu AW320, Germany), meter rule and thread respectively. Photographs were taken using digital camera (Samsung ES95, 16.2 megapixels).

RESULTS

Gross Morphology

Ampulla: The ampullae of the three species are shown on Figures 1, 2 and 3. The terminal wall of the vas deferens on reaching the bladder became thickened and folded to form a structure known as the ampulla**.** It was paired and elongated spindle-shaped in all the three studied species. Structurally, it was the most pronounced in the UR and the least in the OCB. The two ampullae attached to each other through genital fold connective tissues mostly in the UR, followed by OCB and then in the RSB. The broadness of the ampulla was also in that order. The ampullae narrowed and passed under the body of the prostate ventrally to empty into the colliculus seminalis after passing the dorsal wall of the pelvic urethra.

Seminal vesicles (vesicular glands): The seminal vesicles (vesicular glands) of the two species are shown on Figures 1, 2 and 3. The seminal vesicles (vesicular glands) were paired accessory sex glands located on the floor of the pelvis, lateral to the ampulla of vas deferens and the neck of the bladder. Each of the vesicles was connected with the pelvic urethra in the region of the bladder by means of a main excretory duct opening into the colliculus seminalis. They were well developed in both the UR and RSB but larger in the former and absent in the OCB. In these two species, seminal vesicles were markedly lobulated, dark-greyed in color and were the largest of the accessory glands. Though curved in both, they were however more pronounced in the UR in which each pair presented a lateral central depression. In the UR, the dorsal portions were oval in shape and extended laterally up to a reasonable distance of the distal portion of the ampullae while in the RSB, it was knobby in appearance and extended laterally just a short distance of the distal portion of the ampullae.

Prostate gland: The prostate glands of the three species are shown on Figures 1, 2 and 3. The prostate was a single, compound tuboalveolar gland. The gland consisted of only disseminate or scattered parts extending along the pelvic urethra in the UR and RSB. In the OCB, the prostate gland was the largest accessory sex gland. The gland consisted of a dorsal body and a ventral pars disseminate overlying the prostatic urethra. The body of prostate was entirely intra-pelvic, being situated on the dorsal aspect of the urethra and overhanging the neck of the urinary bladder. It was massive and discoid in shape, soft and greyish in color. The cranial two thirds were almost free, while the caudal third was fused with the prostatic urethra. The prostatic urethra was short (3-5 cm). The parenchyma of the body of prostate was observed to be gradually delineated by a thin band originating from the internal aspect of the urethral muscle, thus forming a disseminate portion confined mainly to the prostatic urethra. Caudally, it was continuous with the glandular pelvic urethra.

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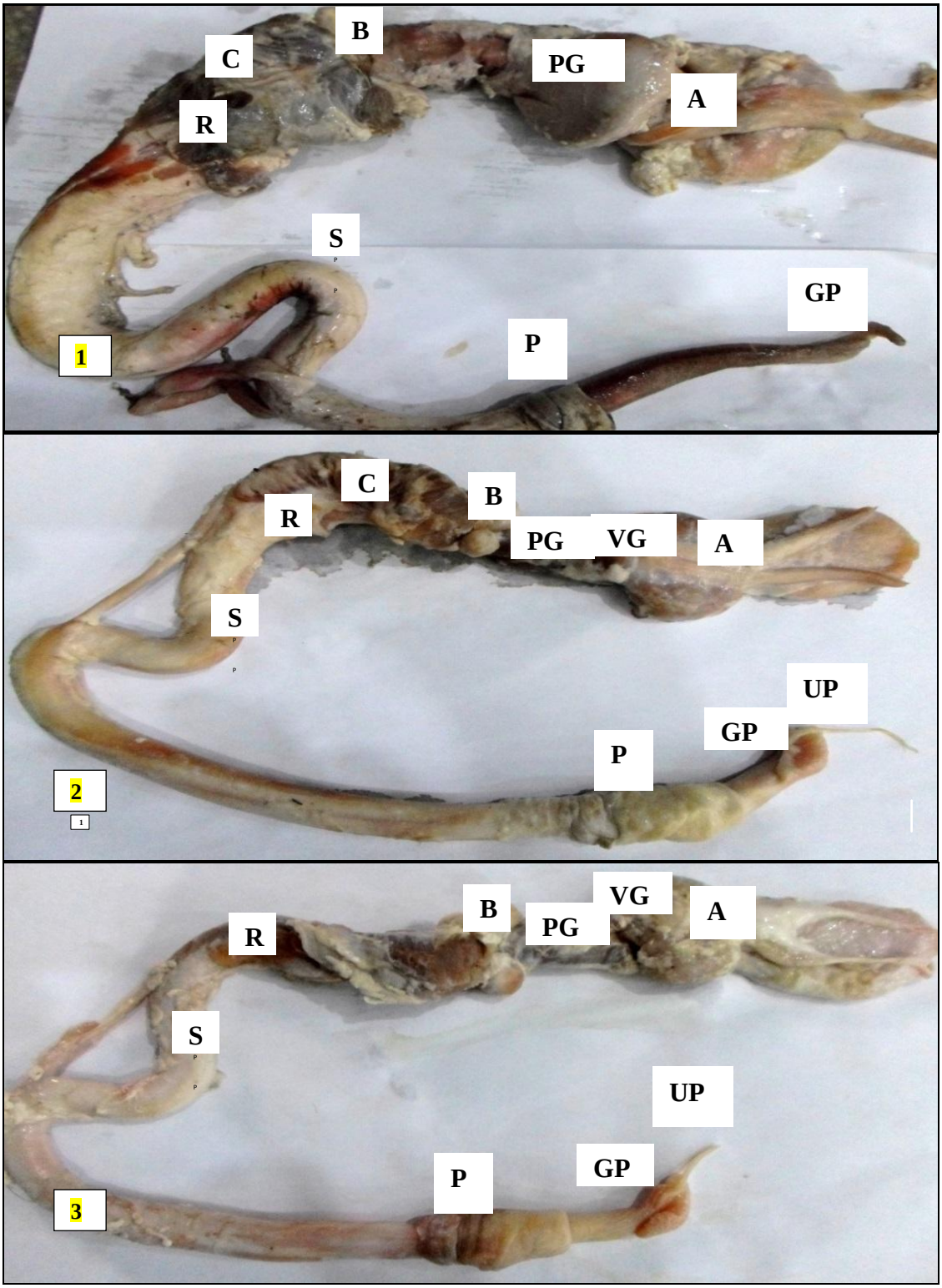
Bulbourethral (Cowper's) glands: The bulbourethral glands (Cowper's glands) of the three species are shown in Figures 1, 2 and 3. The bulbourethral (Cowper's) glands were paired glands located around the pelvic urethra near the ischial arch. In the OCB, they were found on the dorsolateral aspect of the pelvic urethra above the ischial arch with a thick interglandular septum between them and almond in shape. In the RSB, they were found in the same area as in the OCB, though with a relatively less space. In the RSB, they were oval in shape and pea in size. In the UR, the glands were found entirely on dorsal roof of the ischial arch with a very limited space between the pair. Although in all the three studied species, they were partially covered by the urethral and the bulbocavernosum muscles, they were more superficial in UR than in the remaining two species. The glands of all the studied species were covered externally by muscles and internally by a thick layer of dense tissue.



Figures 1, 2 and 3. Photographs of male reproductive tracts of One-Humped Camel Bull (1), Uda Ram (2) and Red sokoto Buck (3), showing ampullary gland (A), vesicular gland (VG), prostate gland (PG), bulbourethral gland (B), cavernosus muscle (C), root of the penis (R), sigmoid flexure (S), prepuce (P), glans penis (GP) and urethral process (UP)

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Gross morphometry

The results on gross morphometry are represented on Tables 1 and 2.

Ampulla: The results of the mean ampulla lengths of OCB, UR and RSB showed that the means were significantly different (P≤0.05). The mean ampulla length value of 19.40±0.74 cm in OCB was significantly (P≤0.05), the highest value in the three species, followed by that of UR (7.38±0.63 cm) and least in RSB (4.17±0.33 cm).

The results of the mean ampulla weights of OCB, UR and RSB showed that the means were significantly different (P≤0.05) from one another. The mean ampulla weights value of 10.92±0.12 g in OCB was significantly (P≤0.05), the highest value in the three species, followed by that of UR (4.35±0.33 g) and least in RSB (1.30±0.15 g).

Seminal vesicles (vesicular glands): The results indicated that there was a significant difference (P≤0.05) between the mean vesicular gland weights of these two species. The mean vesicular gland weight of UR (7.78 ± 0.97 g) was significantly (P≤0.05) higher than that of RSB (5.47 ± 0.58 g). There was no significant difference (P>0.05) between mean vesicular gland lengths of the two species.

Bulbourethral glands: The results of the mean lengths of bulbourethral gland showed that the means were significantly different (P≤0.05) in the three studied species. The mean length of bulbourethral gland in OCB (3.74±0.11 cm) was significantly (.05) higher than those of UR (2.00±0.18 cm) and RSB (1.50±0.29 cm). However, there was no significant (P>0.05) difference between the mean values of UR and that of RSB.

The results of the mean bulbourethral gland weights of OCB, UR and RSB showed that the means were significantly different (P≤0.05). The mean weight of bulbourethral gland of 7.58±0.41 g in OCB was significantly (P≤0.05) higher than those of UR (2.60±0.25 g) and RSB (2.40±0.21 g). However, there was no significant (P> 0.05) difference between the mean values of UR and that of RSB.

The results of the mean bulbourethral gland diameters of OCB, UR and RSB showed that the means were significantly different (P≤0.05). The mean bulbourethral gland diameter of 0.83±0.17 cm RSB was significantly lower compared to those of OCB (2.46±0.18 cm) and UR (2.58±0.22 cm). However, there was no significant (P> 0.05) difference between the mean values of OCB and that of UR.

Table 1. Mean weights and lengths of accessory sex glands of the One-humped Camel Bull, the Uda Ram and Red Sokoto Buck

Parameters	OCB	UR	RSB
Ampulla length (cm)	19.40±0.74[a]	7.38±0.63[b]	4.17±0.33[c]
Ampulla weight (g)	10.92±0.12[a]	4.35±0.33[b]	1.30±0.15[c]
Bulbourethral gland length (cm)	3.74±0.11[a]	2.00±0.18[b]	1.50±0.29[b]
Bulbourethral gland weight (g)	7.58±0.41[a]	2.60±0.25[b]	2.40±0.21[b]
Bulbourethral gland diameter (cm)	2.46±0.18[a]	2.58±0.22[a]	0.83±0.17[b]

[a, b, c] Mean ± SEM within the same row without the same superscript letters are significantly different (P≤0.05) from each other; OCB: One-humped Camel Bull, UR: Uda Ram and RSB: Red Sokoto Buck.

Table 2. Mean ± SEM weights and lengths of vesicular glands of the Uda Ram and the Red Sokoto Buck

Parameters	UR	RSB	P-value
Vesicular gland weight (g)	7.78 ± 0.97	5.47 ± 0.58	0.004[*]
Vesicular gland length (cm)	4.22 ± 0.49	3.33 ± 0.60	0.993[NS]

[NS] Not significant (P> 0.05), *Significant (P≤0.05).

DISCUSSION

The morphology of accessory sex glands as obtained in this study is in agreement with that of Ali et al. (1978) in one-humped camel and that of Khalaf and Merhish (2010) in small ruminants. The presence of the ampullae in OCB as found in this study agrees with the findings of Ali et al. (1978) who reported that the terminal end of vas deferens is markedly thickened to form an ampulla. The result is however contrary to the report of Mukasa-Mugerwa (1981) who said that there is no confirmation as to whether the dilation at the end of the vas deferens is in fact the ampulla or not.

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The result on the ampullae of UR and RSB that were found to be narrow and located ventrally under the body of the prostate, was in agreement with the report of Cunningham (2002) in small ruminants.

Absence of vesicular gland in OCB as found in this study was previously reported by Ali et al. (1978) in one-humped camel. The presence of paired vesicular glands in UR and RSB and their location on the floor of the pelvis, lateral to the ampulla of ductus deferens and the neck of the bladder agree with previous report of Bone (1988) in small ruminants. The anatomical description of the vesicular gland in this study is similar to what was described by Khalaf and Merhish (2010) in small ruminants. The prostate gland of OCB consisted of a dorsal body and a ventral pars disseminate overlying the prostatic urethra. The finding agrees with those reported by el-Wishy et al. (1972) in one-humped camel, who described a corpus prostate and pars disseminate. The finding of small pars disseminate in OCB in this study, agrees with the report of Ali et al. (1978) in one-humped camel. However, it differs from its homologue in the bull (Kainer et al., 1969), ram (Aitken, 1959) and boar (Aitken, 1960), where it extends along the entire length of the pelvic urethra and contains abundant mucous units. The prostate gland in bull, as described by Nickel et al. (1973) to be S-shaped, irregular and elongated in form, often bent on itself, differs from the results of this study in UR and RSB. The results obtained here also disagree with the report of McDonald (1980), who reported that the two lateral lobes of prostate in sheep are well developed and closely distributed along the pelvic urethra. However, the results obtained here, agree with the finding of Dyce et al. (2010) in small ruminants. This difference could be due to difference in species as well as breeds.

The bulbourethral glands were paired and located around the pelvic urethra near the ischial arch. Similar findings were reported in one-humped camel (Ali et al. 1978) and in sheep and goat (Nickel et al.1973; Getty, 1975). Contrary to the present findings of an almond-shaped bulbourethral gland in ram, Roberts (1971) reported that the bulbourethral gland is much larger and consists of two lobes that are cylindrical in sheep. In addition, Cunningham (2002) stated that the bulbourethral gland in bull and horse are enclosed in bulbospongiosum and it is spherical or ovoid in shape. This difference might be due species or breed variations.

The mean length of ampulla obtained in this study was 19.40±0.74 cm in OCB. This is higher than 18 cm previously reported by Ali et al. (1976) in one-humped camel. The mean length of ampulla in UR found in this study was 7.38±0.63 cm. This value is within the range of 6-8 cm previously reported in sheep and goat by Bone (1979). However, the mean length of ampulla in RSB reported in the present study was outside the range. It is however, close to the range of 4.87-4.98 cm earlier reported by Khalaf and Merhish (2010) in ram and buck. This difference might be due species or breed variations

The mean weight (2.40±0.21 g) of bulbourethral gland in RSB as obtained in this study is similar to the reports of Hemeida (1985) who reported a range weight of 2-2.5 g in Balady bucks. However, bulbourethral gland mean diameter of 0.83±0.17 cm obtained in the study differs from his report. This difference may be attributed to genotype.

CONCLUSION

The results showed that the studied animals exhibit some similarities and interesting morphological differences in gross morphology and gross morphometry of their accessory sex glands. The basic gross morphological and gross morphometrical studies done here, have established a comparative baseline data for the accessory sex glands in these animals. It is expected that the results will guide further researchers on the accessory sex glands of these domestic animals.

Competing interests

The authors declare that there are not significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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Cattle Fattening, Constraints and Marketing System in North Western Ethiopia

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ABSTRACT

A study was conducted from January to May, 2015 with the objective to assess cattle fattening and marketing system in north western Ethiopia of north Gondar zone by interviewing 120 cattle fattener households selected purposively. 40% of household source of income were livestock production. Half of the respondents selecting cattles for fattening were red coat color and 80% of them were castrating male animals before the commencement of fattening. Fattening length and age for oxen were 3 months and 7 years old, respectively. From the total of respondents the major sources of feed used for cattle fattening were bean straw (26.67%), nug cake (23.33%), chick pea (16.67%), wheat bran (13.33%), barely straw (10%) and teff straw and hay (3.33%). Decisions on end of cattle fattening period were considering on rate of live weight change (56.67%). Market of fattened animals was during main holidays. The price was highest from February to June, whereas low from September to January. Marketing of beef cattle practiced by bargaining and farmers, cattle traders, whole sales and retailers were involved. The maximum and minimum price of fattened cattle in the dry and wet season was 20000 ETB, 10000 ETB and 13000 ETB, 8000 ETB, respectively. Lack of capital (40%) were the main constraint to begin cattle fattening and other constraints were shortage of feed and water, insufficient land, occurrence of disease and lack of awareness in order of importance 26.67%, 16.67%, 10%, 6.67%, respectively. Therefore, from the present study, it can be conclude that cattle fattening in north western Ethiopia of north Gondar zone is one of the potential strategy to improve the livelihood of the family and had a good potential of market flow.

Key words*:* Cattle fattening, Constraint, Marketing

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INTRODUCTION

Global production of beef and veal is forecast at 59.0 million tons, up modestly from the previous year (USDA, 2016). World meat production is anticipated to record a modest expansion in 2015 to 318.7 million tonnes, 1.3 percent, or 4 million tonnes, above 2014 (FAO, 2015). And also growth in meat trade is projected to decelerate compared to the past decade. Globally almost 11% of meat output will be traded. Meat prices reached record levels in 2014, driven mainly by an increasing beef price (OECD-FAO, 2015). Ethiopia is endowed with largest livestock production, which ranks first in Africa and tenth in the world, it has much to gain from the growing global markets for livestock products (CSA, 2013). Livestock is an integral part of Ethiopia's agricultural sector and plays a vital role in the national economy. At present, livestock contributes about 20% of the Growth Domestic Product (GDP), supporting the livelihoods of 70% of the population and the sub sector also account 11% of annual export earnings (SPS-LMM, 2010). According to the report of BoFED (2004), the agricultural sector in the Amahara region contributed nearly 64% to the regional GDP between the periods 1994 to 2001. It is also known that Ethiopia is characterized by a high livestock population with low productivity of animal products, in terms of conventional products such as meat and milk. Despite the large number of livestock, there has been a decline in national and per capita production of livestock, livestock products, export earnings from livestock and per capita consumption of food from livestock (CSA, 2013). Among exports of livestock products, skins and hides have the largest share of exports followed by live animals (MEDAC, 1998; FAO, 1999). Crop mixed farming system is the predominant farming systems in the highlands of Ethiopia. They inhabit nearly 90% of the human population and 70% of the livestock population of the country (Mohamed-Saleem and Abate, 1995). Due to the rising of population growth, lack of land pushing many farmers either to intensify the cropping system or diversify the system using other integrated activities.

Livestock and meat products have been among the fastest growing components of the global agriculture and food industry. This growth reflects not only increasing demand for meat as global incomes have risen, but also improved efficiencies in production, processing and transportation declining real feed prices (Morgan and Tallard, 2015). Global meat trade is forecast to expand at a moderate rate of 1.7 percent in 2015, to 31.2 million tones, a significant slowdown from the 3.1 percent registered last year (FAO, 2015). Meat production and consumption is important in the Ethiopian

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economy and ruminants contribute over 3.2 million tons, representing over 72% of the total meat production (Belete et al., 2010). Even if, the Cattle population in the majority of tropical country is higher, there is a strong unsatisfied demand, due to the increment of population growth in the majority of tropical countries, for milk and meat (FAO, 2015). However, the actual consumption is seriously restricted by the low purchasing power of the majority of the consumers, for whom retail prices are already too high. At the other extreme, the producer is in a difficult position and the course taken, notably for beef, does not allow to envisage the introduction of more intensive techniques, the only ones which would enable an increase in production when the limits of expansion of the pasture area are reached (Reag and Lipper, 2012).

In north Gondar there was estimated to have less supply of crop-residues, there may be mishandling and lack of awareness about crop-residue improvement (NGZARDO, 2014). As a result, utilization efficiency of the residues was low. Lack of proper selection of fattening cattle, fattening practice, lack of market information and also poor managements in relation to feeding system, healthcare, housing etc. reduced the performance of cattle fattening (NGZARDO, 2014). Hence, the producer may not get reasonable benefit from their fattening activity unless appropriate improvement strategies have to be introduced. In addition to this, detail studies on sources of feed available for cattle fattening, constraints and marketing system of cattle in the area was not further studied. Thus, on the basis of this background, the objective of this study was, to assessing the cattle fattening practices, constraints and marketing system of cattle fattening in north western Ethiopia of north Gondar zone.

MATERIAL AND METHOD

Description of the study area

The study was conducted in north western Ethiopia of north Gondar zone. The area is located at a distance of 737 km north of Addis Ababa. The area lies between an altitude of $12^{o}35'60"N$ and longitude of $37^{o}28'\ 20"E$ and has an elevation of 2300 masl. Gondar has a varied landscape, dominantly covered with ragged hills and plateau formations. The annual average temperature was $19.7^{0}c$ and its annual rainfall was 1772 mm. It could be categorized under winea dega climatic zone. The area is also classified mainly in to two seasons, the wet season, from June to September and the dry season from October to May.

Data collection and sampling techniques

Data to be collected were employed by using purposive sampling techniques. A total of 120 respondents were purposively selected. Both primary and secondary data was employed. The primary data were obtained through structured questionnaire and semi structured interview with the owner of cattle fattener. Secondary information also utilized from Gondar Woredas and zonal Agricultural and Rural Development Office (ARDO).

Statistical analysis

The data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) software, version 16.0. Descriptive statistics such as frequency distribution and percentages were used.

RESULTS AND DISCUSSION

Socioeconomic description of households

Socioeconomic descriptions of households are presented in Table 1. From the total of respondents majority of the household heads in the study area were married and among them 80% of males were the responsible person on fattening activity. 66.67% of fattening participants were between the ages of 31-40 and 46.67% of respondents their educational level were completed high school. The major occupation of households in the study area was identified as livestock production, crop and livestock production and Trade, 40%, 26.67%, 23.33% respectively. The average age of the households involving on fattening practices were similar with that of Hossain et al. (2002) which was the average age of the farmers involving on fattening in a range from 27 to 40 years old.

Cattle fattening practice in the study area

Selection criteria of fattening cattle: Parameters of cattle selecting and castration for fattening purpose are indicated on figure 1. From the total of household respondents half of fatteners were select the red coat color (50%) cattle for fattening purpose and bulla (mixed color), white, black were 26.7%, 13.3%, 10% respectively. Castration of animals was also another criterion for fattening in the study area. Accordingly, 80% of the respondents castrate their animals' before fattening while the remaining 20% of the respondents were not recommending for castration. Majority of

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the respondents were fatten only male cattle (83.33%), where as 16.67% respondents were kept both male and female animals in the study area. According to the finding of the present study one of the criteria's of fatteners to select animals before fattening were based on the animals coat color however, this criteria's were not agreed with that of the report of Belete et al. (2010) almost all traders do not take coat color as a criterion for selection of beef animals.

Table 1. Socioeconomic description of households in north Gondar zone Ethiopia, 2015

Variables	Respondents	%
Sex		
Male	96	80
Female	24	20
Age (year)		
20-30	36	30
31-40	80	66.67
Above	4	3.33
Educational level		
1-4	16	13.3
5-8 (elementary)	24	20
9-12 (secondary)	56	46.67
Above (higher education)	24	20
Source of income		
Livestock production	48	40
Crop and livestock production	32	26.67
Trade	28	23.3
Other	12	10

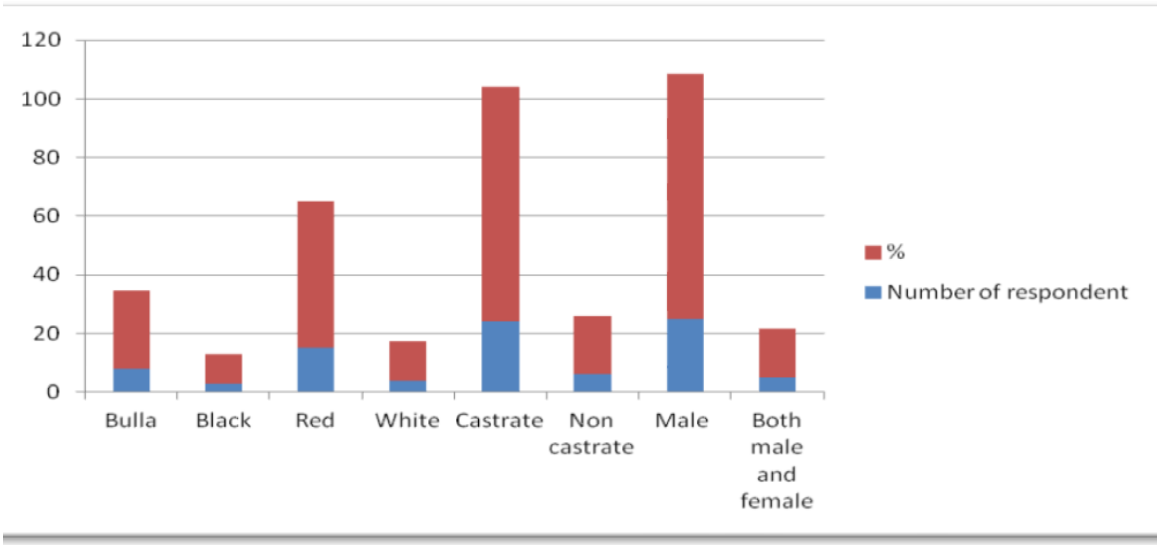

Figure 1. Parameters of cattle selecting and castration for fattening purpose in north Gondar zone Ethiopia, 2015

Age and duration of cattle fattening

Length of time and age of fattening cattle in the study area are indicated in Table 2. The fatteners in the study area select and fatten mature and much older animals (7-8 years old) (53.33%), the durations for fattening end was usually 3 months (83.33%), 3.5 months (13.33%) and Four months (3.33%) of the respondents. Duration of feeding was dependent on feeding method that the cattle being fattened with pure feedlot finished within three months of feeding length.

Cattle age of selection for fattening was similar with that of the report of Belete et al. (2010) which was smallholder farmers commonly fatten mature and therefore much older animals (five to seven years old) for short durations (usually three months). The present findings was also in agreement with that of the report of Takele and Habtamu (2009) and BoARD (2004) who reported that cattle feeders fed cattle usually for four months in southern and northern Ethiopia, respectively. This is also supported by the report of cattlemen's beef board and national cattlemen's beef association (2009) which was most beef cattle spend approximately four to six months in a feedlot just prior to harvest where they are fed a grain-based diet. However, this study was in contrast with that of Habtemariam (2000), farmers in east Ethiopia fed oxen for more than one year which was also significantly exceeds the average fattening length in southern parts of Ethiopia.

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Sources of feeds and feeding system

Sources of feed for fattening cattle in Gondar town are indicated on Table 3. From the total of household respondents the feed sources which was used for fattening purpose were bean straw, nug cake, chick pea ,wheat bran, wheat straw and teff straw, 26.67%, 23.33%, 16.67%, 13.33%, 6.67% and 3.3% respectively. The present finding was similar with the report of Takele et al. (2009) in southern region and Belete et al. (2010) in Amhara region of Ethiopia. Major feed resources used as a basal diet for fattening cattle were crop residues. However, according to the report of Shapiro (2016), one of the challenges of cattle fattening were feed shortage such as poor quality of grazing lands, A need for greater knowledge on the use of crop residues and Poor availability of concentrates and feed supplements when needed.

Methods to decide finishing period of fattening cattle

Decisions on end of cattle fattening finishing period are indicated on Figure 2. From the total of respondents deciding finishing period of fattening cattle in the study area were based on considering rate of live weight change (56.67%), while 40% of them were anticipated current and future prices, and others by calculating feeding length (3.33%).The present study were in line with Shitahun (2009) ending of cattle finishing period was decided by considering live weight change of fattening cattle with visual observation based on their feed intake (84.97%) and by anticipating the current and future price (15.03%).

Table 2. Length of time and age of fattening cattle in north Gondar zone Ethiopia, 2015

Duration	Respondents	%
3 month	100	83.3
3.5 month	16	13.33
4 month	4	3.33
Total	120	100
Age of fattening cattle in year		
6 to7	32	26.67
7 to 8	64	53.33
Above	24	20
Total	120	100

Table 3. Sources of feed for fattening of indigenous breed cattle in north Gondar zone Ethiopia, 2015

Sources of Feed	Respondents	%
Bean straw	32	26.67
Wheat straw	8	6.67
Barely straw	12	10
Chick pea	20	16.67
Nug cake	28	23.33
Wheat bran	16	13.33
Teff straw and hay	4	3.33
Total	120	100

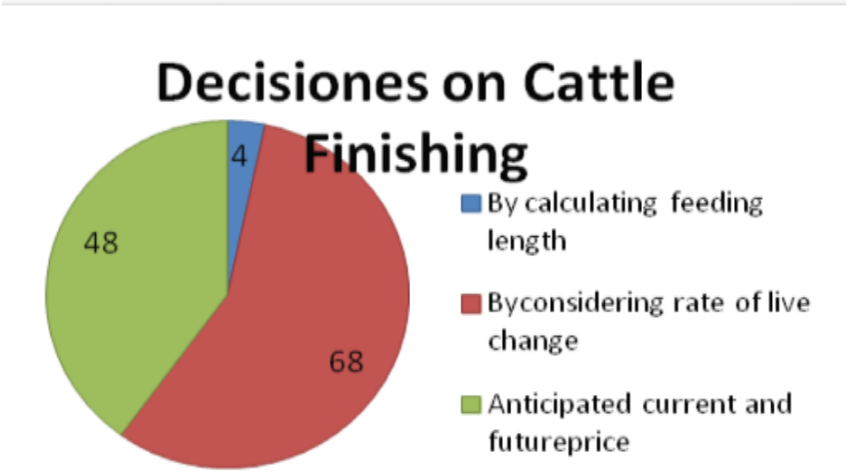

Figure 2. Decisions on end of cattle fattening finishing period in north Gondar zone Ethiopia, 2015

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Season of cattle fattening and marketing

Results on season of cattle fattening and marketing in Gondar town are indicated on Figure 3. From the total of household respondents 60% were participated fattening at the time of holiday however, 40% of them were involved on the time of non holiday and 66.67% of cattle fattening were starting from mid February up to June, this may be due to the price of cattle for fattening is low in the market. In some extent cattle fattening activity was starting from September to January (33.33%), targeting to deliver fattened cattle for Meskel and Christmas. The price of beef cattle in Gondar town after fattening on average was 15000 ETB. The maximum and minimum price in the dry and wet season was 20000 ETB, 10000 ETB and 13000 ETB, 8000 ETB respectively.

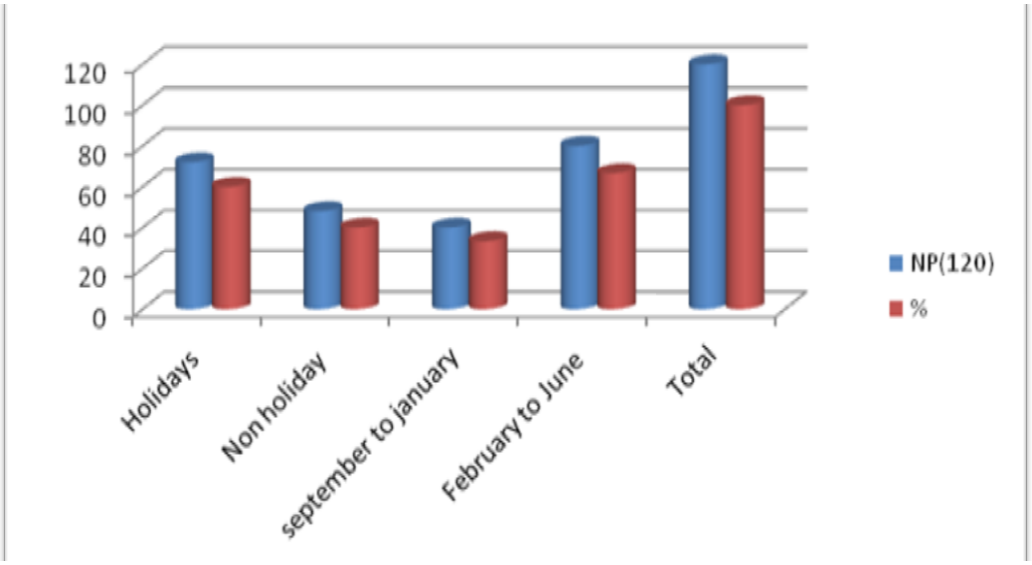

Figure 3. Seasons of cattle fattening and marketing in Gondar town in north Gondar zone Ethiopia, 2015

Season of cattle fattening were agreed with the reports of Takele et al. (2009) which was studied at Welaita, who reported that cattle fattening was a seasonal operation with a peak from June to September and this is governed by seasonality pattern of feed availability and main holidays. This low extent of cattle fattening activity for meskel and Christmas market was due to the presence of enough fresh grass feed, supply of fattened cattle is greater than the demand and decreased purchasing price of fattening cattle. It was also associated with low market demand for fattened cattle because of the custom of the local people preference towards consumption of fattened sheep and goats instead of fattened cattle during Christmas. Starting from July up to August, cattle fattening was totally absent in the study area. However, the market price of fattened cattle was in contrast with Belachew (2004) and Takele and Habtamu (2009) the market price of fattened cattle in northern parts of Ethiopia was highest from September to April. The reason for this might be due to the availability of the main holidays in September (meskel) and December (Christmas). Marketing system of fattening cattle was undar developed due to the knowlege, performance of fattener and less attension given by livestock subsector. In addition to this Habtemariam (2009) also stated that the price of cattle lower during the dry season due to the shortage of feed and water.

According to the present study the fatteners sell their fattened cattle by visual estimation negotiation with customer in the study area. The price of fattening cattle depends on weight and age of the animals. Hence, fattening more closely resemble to fattening of culled cows, however, the fattener decide the end of finishing period of fattening cattle by considering rate of live weight change in the study area. However this was in agreement with the findings of Alemayehu (2003) who reported that marketing of livestock was not determined on the basis of weight and which was unfavorable marketing system and discourages price on the producers.

Marketing channels of beef cattle

The channels of beef cattle marketing found in the study area are shown in Figure 1. Before and after the Holidays, animals are taken to local market for selling, on market days. Buying and selling are completed through bargaining practice. In the process of cattle marketing farmer, whole seller, trader and butchers are involved.

The report of the present study were agreed with that of Rashid (1969) marketing channel referred to the sequential arrangement of various marketing intermediaries involved in the movement of products from producer to consumers. Participants in beef cattle was also in line with that of Belete et al. (2010), participants in beef cattle marketing in fogera, are producers, middlemen or brokers, traders and consumers however, butchers and hotel owners directly buy beef animals directly from the producers.

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Major constraints affecting cattle fattening in north western Ethiopia

Factor affect cattle fattening in the study area are indicated on Table 4. Fatteners suggest different constraints that hindered the performance of cattle fattening activity in the study area. Lack of initial capital, shortage of feed and water, insufficient land, occurrence of disease and lack of awareness (40, 26.67, 16.67,10 and 6.67% respectively).

The present study was similar with the finding of Belete et al. (2010) reported that the critical constraints to improve dairy and beef cattle production in the Woreda are feed shortage, high disease prevalence, shortage of improved dairy breeds, poor extension service, Artificial Insemination (AI) and veterinary services, lack of working capital, marketing problems for dairy and beef products during the long fasting periods, lack of market information system and lower purchasing power of the local consumers in Amhara region of Ethiopia. However, it was in contrast with Getnet (2003) reported that feed quality and quantity is the main limitation to animal production in Ethiopia.

Table 4. Factor affect cattle fattening in north Gondar zone Ethiopia, 2015

Factors	Respondents	%
Lack of initial capital	48	40
Shortage of feed, water	32	26.67
Insufficient land	20	16.67
Occurrence of disease	12	10
Lack of awareness	8	6.67
Total	120	100

;8

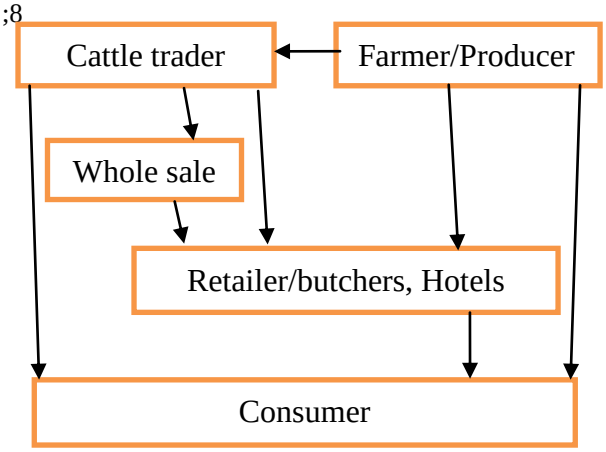

Figure 4. Flow chart illustrating marketing channel of beef cattle in north Gondar zone Ethiopia, 2015

CONCLUSION

The overall results of the present study showed that the major occupation of households in the study area is on livestock production. Fatteners using oxen for fattening purpose are old, red coat color and castrated. The feed sources used for cattle fattening are bean straw, nug cake, chick pea, wheat bran, barely straw and teff straw and hay. The major fattening practice is starting from mid February up to June. Their feed sources were crop residues and industrial by-products. The maximum price recorded during the dry season and the lowest price is in wet season. The major constraints for fattening practices is lack of initial capital, shortage of feed and water, land shortage, occurrence of disease and lack of awareness. Generally**,** cattle fattening practices is one means of household livelihood improvement in north western Ethiopia of north Gondar zone. Based on this information, it is recommended that, the government should give due attention on market channels of fattened animals in north Gondar zone. Extension policies and strategies on fattening practices, feed improvement strategies, credit service, training and extension service (advice on beef selection, feeding, health care and market information) and further researches on reproductive performance of fattening cattle and carcass quality related to feeding in north Gondar zone should be conducted.

Competing interests

The authors have no competing interests to declare.

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Diagnosis and Therapeutic Management of Tetanus in Female Buffalo Calf at Tandojam, Sindh, Pakistan

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ABSTRACT

A female buffalo calf with wound on left leg just below the knee joint suffering from high and persistent fever, anorexia, difficult mastication and urination, stiffness in neck muscle and with some degree of bloat was brought to department of veterinary medicine faculty of animal husbandry and veterinary sciences, Sindh agriculture university, Tandojam, Pakistan, and admitted. The calf was diagnosed to be suffering from tetanus based on clear cut symptoms of high fever, stiff muscles, urine retention and fixed jaws. The Graham's staining of the fresh smear revealed gram+ve rod shape bacteria that appeared like drumsticks. Furthermore, the *Clostridium tetani* was cultured and isolated from the deep necrotic tissue of the wound. The calf was treated with high doses of procaine penicillin, anti-tetanus serum, sedative, meloxicam and intravenous fluid electrolyte therapy (Dextrose 5%). The calf was feed through stomach tube and the urinary catheter was administered to ease out the problem of urine retention. After continues therapeutics management, the calf recovered in two weeks.

Key words: Buffalo calf, *Clostridium tetani*, Diagnosis, Therapeutic management

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INTRODUCTION

The tetanus and gas gangrene are rarely encounter in adult population; injury being the single most common cause of these infections in adulthood. Neonatal tetanus (NT) is used as an indicator of overall tetanus burden in study area (2015). Tetanus's disease causing organism is the bacterium (*Clostridium Tetani)* that is the similar family of organisms which causes blackleg (Lewis, 2015). During sporulation, the vegetative form of the organism develops into a spore form, giving a characteristic drumsticks appearance in blood smear. The spores of this organism are very resistant to disinfectants, for example acidified phenol takes about two hours to kill them. Moreover, in spore form they can survive for many years in soil (Radostits et al., 2007).

It is a sporadic and ubiquitous disease that occurs throughout the world (Smith, 2002). Being anaerobic bacteria the tetanus bacilli do not multiply in normal tissues or even in damaged tissues with the same oxidation - reduction potential to that of normal blood. Both necrosis and low oxygen tension in injured tissues provide a sufficient anaerobic environment for multiplication of bacteria producing toxins (Bizzini, 1986). Two types of toxins, tetanospamin and hemolysin are generally produced by *Clostridium tetani* (Montecucco and Schiavo, 1995). Tetanus is an infection categorized by muscle spasms. In the most well-known type, the spasms begin in the jaw and after that progress to the rest part of the body. These spasms remain a few minutes each time and occur repeatedly for three to four weeks *(*Atkinson et al., 2012). This bacterium is generally present in feces of animals or in soil contaminated with these feces, and enters to the body of the susceptible animals through wound contamination, in calves the wound due to castration is also, one of the major causes for entrance of bacteria to the body (Connor, 1993; Radostitset al., 2007). Tetanus in young calf due to contamination of umbilicus after parturition can also, induced mortality (Suleman, 1982). An injured area with low oxygen tension it multiplies leading to necrosis of the deep tissues providing a much favorable environment for persistence of bacteria, the bacterial cell thus ruptures and neurotoxins are released the from where the toxins enter to the motor or sensory nerve. If the amount of toxins is high they get entry to the blood and lymphatic vessels and reach to Central Nerves System (CNS) where they block the release of neurotransmitters causing all groups

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of the muscles to contract simultaneously (Montecucco and Schiavo,1995). The initial clinical signs such as stiffness of neck muscles with extended head mostly appear after 24 hours. Other clinical signs include twitching and tremors of the muscles, firmly fixed jaws (Lock jaw), protrusion of third eyelid, and lameness with alert expressions, hyperesthesia, erected ears and dilated nostrils. Bloat can also, occur because the rumen stops working. Later signs include collapse, lying on side with legs held stiffly out, spasm and death (Smith, 2002; Radostits et al., 2007).

CASE REPORT

A six months old female buffalo calf was brought to the clinic in department of veterinary surgery, faculty of animal husbandry and veterinary sciences, Sindh Agriculture University Tandojam Pakistan. The calf had fallen in farm and sustained an injury on his left leg just below the knee joint. The surgeon cleaned the wound and inserted a simple suture, none of the tetanus anti-toxoid and antibiotics were given. According to the farmer, a horse and donkey in his farm were kept for bringing fodder in the same area where the animals are reared. After two days, the calf was depressed and there was some degree of lameness. No evidence of fracture was noted after performing an X ray. Therefore, the case was referred to department of veterinary medicine tandojam Pakistan, the rectal temperature of the calf was higher (106.8^0F) and persistent at the same degree after giving antipyretics. On the other hand there was problem with mastication and urination, with some degree of bloat which was not corrected after use of carminative mixture. Keeping in view the poor prognosis after symptomatic treatment the patient was admitted. On next day, the rectal temperature of the calf was persistent at 107^0F, and the calf was depressed and anorexic. There was stiffness of the neck and jaw muscles, both the ears were erected with alert and anxious expressions. After thirty minutes there was generalized increase in muscle stiffness with lock jaw, prolapse of the third eyelid and stiffness of the hind legs causing staggering in gait. Additional signs exhibited by the animal included dilation of nostrils, hyperesthesia, drooling of saliva from mouth and urine retention.

Diagnosis

On the basis of the above mentioned clinical signs exhibited by the calf, the case was subjected to laboratory diagnosis. The wound area was palpated and there was some pus containing swelling. Therefore, the suture was opened and a fresh smear was prepared from the deep necrotic tissues. The tissue sample from the same site was also, taken for culture and isolation of *Clostridium tetani*.

Smear preparation

In order to prepare a fresh smear, a clean glass slide was taken and touched with the deep tissues of the site. The slide was then immersed in absolute alcohol to fix and then air dried at room temperature (Congera and Lucile, 2009).

Graham's staining technique

First the prepared slide was flooded with basic crystal violet for one minute and washed with water. Then Graham's iodine was applied for one minute and washed with water. After this the slide was decolorized with alcohol for 15 seconds and washed with water. At last the counter stain "safranin" was applied for 30 minutes, washed with water and dried. Using the compound microscope the slide was examined under oil immersion lens (100×) as used by (Ali et al., 2005).After detailed examination of the slide under microscope, a violet colure Graham +verod shaped bacteria was appeared like drumsticks.

Culture and isolation of bacteria

In order to confirm the causative bacterium, it was isolated for which the bacteria were first cultured on blood agar medium. To prepare the blood agar, a fresh defibrinated sheep's blood (5 to 10 ml) was used. The nutrient agar was cooled up to 45^0C and autoclaved for 24 hours. After that, it was mixed with the required amount of sheep's blood. The prepared medium was poured into sterilized Petri dishes and incubates for overnight. Streak plate method was used to isolate *Clostridium tetani*. A drop of pure culture was placed near one end of the petri dish; the platinum loop was flamed and then cooled by touching on sterile surface of the medium. The petri dish was held in left hand within six inch diameter of the flame. Using the sterile lope from the surface of the culture on the edge the bacteria were streaked on half of the plate in parallel position. The lope was again sterilized and cooled, the plate was then rotated at 45^0angle and the remaining ¼ of the petri plate was streaked from the last line. The plate was rotated and streaked the remaining portion of the plate. The streaked plates were then placed in an anaerobic jar and air tight and then incubated in 37^0C in an anaerobic incubator as per procedure of Calvin et al. (1969). After detail examination of the plate, single sized, plate, grayish and translucent colonies with irregular margins were observed. Thin zones of beta hemolysis were also noted around the colonies. On the basis of characteristics of colonies it was confirmed that the causative agent was *Clostridium tetani* (Tina, 2010).

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Treatment

First of all the affected animal was transferred to an isolated room and cotton plugs were applied in both ears to reduce hyperesthesia. The wound was excised widely; all the contaminated tissues were removed and packed with gauzed soaked in hydrogen peroxide. Then multi vitamin and dextrose were administered at 1500 International Unit (IU) intravenously in jugular vein. The sedative (xylase) was administered at the dose of 0.1 mg/kg body weight. After this 30000 IU per kg of body weight of penicillin G procaine was given intramuscularly for 12 days. An I/V injection of ATS (Anti-Tetanus Serum) 1500 IU was also administrated (Bhikane et al., 2005).Chlorpromazine50 mg/kg and meloxicam (Diclostar) 0.6 mg/kg body weight were also used. Intravenous fluid therapy (dextrose 5%) 150 ml/kg body weight was continued for eight days. The feed was given through a stomach tube and urinary catheter was administered to ease out the problem of urine retention.

RESULTS AND DISCUSSION

After continues therapeutic management the calf was capable to stand at 4thday of treatment, while muscle stiffness especially that of neck region was persistent for several days; however the calf was completely recovered at 2nd week. These results corroborate with those reported by Das et al. (2011). The patient in the present case was completely anorexic, therefore the calf was fed through stomach tube, which is fully in line with that of Lombar and Zadnik (2013) while the sedative caused the relaxation of the skeletal and deep body muscles including diaphragm and intercostal muscles that helped in normal respiration. Anti-tetanus serum has the effect to neutralize the toxins and thus prevented the central nerve system. The Meloxicam provided relief from pain and inflammation. These findings are in accordance to that of Radostitset et al. (2007) and Bhikaneet et al. (2005) but the interesting thing in therapeutic management of the present case was use of urinary catheter and feeding of the calf with stomach tube most of the clinicians avoid these things and thus a lot of mortalities may occurs due to severe debility and rupture of the urinary bladder. Furthermore, in the current case penicillin was used as antibacterial. According to Smith et al. (1964) penicillin provide a good prophylaxis against tetanus for injured patients who have not immunized against tetanus.

CONCLUSION

From the current case it is concluded that it is necessary in tetanus to take the history about urination and nutrition status of the animals. If the animal has not urinated for a long time, the urinary catheter should be used to ease out the problem of urine retention. Also stomach tube should be used in case of firmly fixed jaws for feeding of the animals to avoid debility.

Competing interests

The authors have no competing interests to declare.

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Cadmium Bio-Accumulation and the Associated Biomarkers in Edible Frog Species (*Hoplobatrachus Occipitalis)* in Ibadan, Oyo State, Nigeria

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ABSTRACT

The spate of natural emissions and anthropogenic activities has comparatively increased cadmium pollution in recent times. This has also increased the attendant hazardous implication on both the aquatic and terrestrial ecosystems. In this study, a total of 50 edible frog species (*Hoplobatrachus occipitalis*) sourced from the Ogunpa river in Ibadan, Oyo state were sampled. Atomic Absorption Spectrophotometry (AAS) was used for the evaluation of the blood, kidney and liver cadmium level. The frogs were grouped into Below Permissible Limit (BPL) and Above Permissible Limit (APL) groups using the FAO/WHO cadmium permissible level of 0.5mg/kg. 86% of the sampled frogs had blood cadmium level above the permissible limit while the liver and kidney cadmium levels exceeded the permissible limits in all the frogs. The highest cadmium level was detected in the liver (3.02±1.23 mg/kg). The erythrocyte parameters were significantly lower in the APL compared to the BPL group while the leucocyte parameters were higher in the APL than the BPL group. The histopathological lesions were consistent with pathological changes associated with renotoxic, hepatotoxic and reproductive features of cadmium toxicity. The study highlights the elevated cadmium levels in the tissue of the frog as a biomarker of exposure while the haematological and histopathological changes served as biomarkers of effect associated with cadmium toxicity in naturally exposed frogs. It also serves to underscore the importance of frogs as important sentinels of environmental cadmium toxicity, creation of public health awareness for cadmium toxicity and the evaluation of cadmium toxicity in the ecosystem.

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INTRODUCTION

Cadmium is a naturally occurring heavy metal that poses considerable detrimental biohazard effect on the ecosystem and poses severe health risk to human health. According to the International Agency for Research on Cancer (IARC) (2016), cadmium has been classified as one of the heavy metals (along with others like beryllium, nickel and their compounds) as a group one carcinogen. The ubiquitous presence and environmental pollution caused by cadmium has increased considerably over the years due to an increase in anthropogenic activities which utilizes them (Don-Pedro et al., 2004). Cadmium is usually obtained naturally as combined ores along with those of other heavy metals such as zinc, copper and lead. As such, tons of these heavy metals along with the industrial effluents are released during the extraction and smelting of some of these ores (Bernhoft, 2013).

The relatively high resistance to corrosion, low melting temperature, electrical exchange properties and excellent conductivity (thermal and electrical) of cadmium have also comparatively increased the utilization of cadmium in many industrial processes. Cadmium has thus found extensive application in the production of alkaline nickel-cadmium batteries, electronics, electroplating, nuclear fission cores, industrial pigments, alloys and as adjunct impurities in other metals (steel, zinc, lead and copper) (OECD, 1994 and Bernhoft, 2013).

This extensive use and the poor mechanism involved in the solid waste disposal of some of these products after their use have led to the abundance of cadmium in dumpsites and landfills globally. Water sources (groundwater, lakes, streams and rivers) can be polluted by cadmium leaching from industrial and consumer waste. The weathering of cadmium ore rich rock also serves as an example of natural activities contributing to environmental cadmium pollution (Environmental Protection Agency (EPA), 1985 and Morrow, 2001). These anthropogenic and natural activities therefore serve to encourage the mobilization and release of cadmium from non-bioavailable geological matrices into the ecosystem at concentrations far above the natural cycling process (Suru, 2008). The combination of different physical, chemical and microbial activities has been adduced by different researchers to be involved in the breakdown of some of these wastes and thus the release of cadmium into the environment (Leyval and Joner, 2001 and Gadd, 2010). The

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leaching of cadmium from dumpsites and rocks is also comparatively aided by acid rain which can exacerbate this process by enhancing the ease of cadmium release into the soil. This has inexorably led to an increase in cadmium pollution with dire deleterious impact on both the terrestrial and aquatic ecosystem (Worsztynowicz and Mill, 1995).

Cadmium serves no physiological role and despite the biotoxic effect it is not metabolized to less toxic forms in the body of the animal. Cadmium is also capable of accumulating in the tissues of the exposed animals when it is absorbed at a level in excess of the excreted volume (Britton, 1998 and Godt et al., 2006). Cadmium therefore tends to bioaccumulate in living organism with progressive increase in concentrations in biota several orders of magnitude greater than the environmental level. More so, the biomagnification of this metal also progressively increases with each trophic level. Humans, due to their position in the trophic level of the ecosystem, are therefore exposed to an elevated level of cadmium in excess of the environmental level (Wang, 2012 and Croteau et al., 2005).

The toxic bioaccumulation of cadmium in living organisms (plants and animals) has been widely studied as a vital indicator of environmental exposure. This bioaccumulation has been associated with toxic effects such as nephrotoxicity, carcinogenicity, teratogenicity and endocrine disruption (Serafim and Bebianno, 2007; Ikechukwu and Ajeh, 2011). These toxicities have been ascribed to the ability of cadmium to interfere with different enzymatic and biochemical processes in the body. Some of the mode of action employed in cadmium interference with physiological processes include: alteration of enzymatic processes and protein conformational changes. Cadmium is able to elicit these alterations due to the ability of cadmium to displace other metals (Copper, magnesium and zinc) and alter sulfhydryl groups in metalloproteins and metalloenzymes (Ikechukwu and Ajeh, 2011 and Haschek et al., 2013). Cadmium also induces oxidative stress through antioxidant enzymes binding, glutathione depletion, alterations of electron transport chain and metallothionein binding (Stohs and Bagchi, 1993; Hultberg et al., 2001 and Ercal et al., 2001). Other associated mechanism of cadmium toxicity includes: inhibition of heme synthesis (Chauhan et al., 1997; Nogueira et al., 2003 and Schauder et al., 2010), potentiation of apoptosis due to impairment of mitochondrial function (Cannino et al., 2009), oxidative DNA damage and epigenetic DNA changes (Martinez-Zamudio et al., 2011; Luparello et al., 2011 and Wang et al., 2012).

Frogs and toads due to their amphibious nature serve as an important link between the terrestrial and aquatic ecosystem thus making them invaluable as bio-indicator of different environmental contaminants (Hayes et al., 2002 and Unrine et al., 2007). This has also made them important sentinels both for the assessment of the presence and the evaluation of the toxic impact of environmental toxicants (Carey and Bryant, 1995; Loumbourdis et al., 2002; Papadimitriou et al., 2003; Rabinowitz et al., 2008 and Othman et al., 2009). Frogs have been extensively used in different studies as bio-indicators of pollutants accumulation due to their perpetual presence in water which keeps them directly in contact with the pollutants. The relatively thin nature of their skin and their position on the trophic level also makes them prone to persistent exposure to effluents in the water where they reside (Papadimitriou et al., 2003).

The crowned bullfrog (*Hoplobatrachus occipitalis*) is a species of frog in the Dicroglossidae family. It is found in the Sub-Saharan Africa. It lives in many habitats from dry savannahs to disturbed forest, using logging roads and rivers to penetrate deep into lowland forest. It naturally inhabits both aquatic and terrestrial areas therefore making it an excellent sentinel animal (McDiarmid and Mitchell, 2000; IUCN SSC Amphibian Specialist Group, 2014). The crowned bullfrog is often used as important sentinels because they serve as typical representative amphibians that could be used for assessment of environmental toxicity. This along with their categorization under the "Least Concern" or LC category also makes them a suitable candidate as sentinel animals (IUCN SSC Amphibian Specialist Group, 2014). More so they are highly prolific, easy to handle and comparatively economical to use for field evaluation and as an experimental model (Ezemonye and Enuneku, 2012). The sensitivity of frog to cadmium toxicity also makes them an excellent sentinel candidate for the evaluation of biomarkers and evidences of cadmium toxicity in the environment (James and Little, 2003; Rosenberg et al., 2007 and Ezemonye and Enuneku, 2012).

Despite the volume of ecotoxicological research using amphibians in recent times, there is however still a dearth of information on the impact of cadmium toxicity on local African frog species. This study therefore serves to elucidate the bioaccumulation and associated biomarker of effect in the indigenous crowned bullfrog while also highlighting their important role as sentinel for ecotoxicological assessment. The findings from this research could thus serve as an important guide in the use of the crowned bullfrog as sentinels in designing remediation programmes, cadmium toxicity intervention and the assessment of the impact of cadmium toxicity on conservation, ecosystem and public health.

MATERIALS AND METHODS

Study area

This study was conducted in Ibadan (7º23' N, 3º5' E), Oyo State in the south western part of Nigeria. The bullfrogs (*Hoplobatrachus occipitalis*) were sourced from the swamp and marsh of the Ogunpa River, Ibadan, Oyo State, Nigeria. The bullfrogs were captured at night from their spawning sites using net traps and hand nets to prevent injury to them.

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Animals

A total of 50 frogs (26 male and 24 female) were captured and used for this study. The frogs were apparently in good health condition and active. The weight of the sampled frog ranged from 30 – 58 grams. Both male and female frogs were used for this study and sex identification was done using the secondary sexual characteristics (head morphology and vocal cord identification) for the male and the identification of the gonads.

Sample collection

Blood collection: The frogs were initially anaesthetized using chloroform soaked cotton wool in a fume chamber. Blood samples (2 – 3 ml) were then collected by cardiac puncture from the ventricles. 1ml of the collected blood samples was transferred into lithium heparin bottles for complete blood cell count analysis. 1 – 2 ml of blood was preserved for cadmium blood level analysis using atomic absorption spectrophotometry.

Tissue collection: After the blood collection, the frogs were returned into the fume chamber for euthanasia in accordance with the institutionally approved procedures for humane handling of experimental animals. The frogs were afterwards dissected for collection of the tissue samples (liver, kidney, testes and ovaries). 1–2 grams of the liver, the kidney along with the testes or ovaries were collected in buffered formalin for fixation and histopathological sectioning. Some of the liver and kidney were also collected in plain bottles and stored in the freezer for heavy metal analysis.

Sample Analysis

Haematological parameters: Complete blood count and quantitative analysis of the haematological parameters were done immediately to prevent haemolysis and associated preanalytical errors. The haematological parameters considered in this study include the red blood cells counts, haemoglobin concentration, packed cell volume, platelet count, total white blood cell count and the absolute heterophil, eosinophil, basophil, monocyte and lymphocyte count as described by Jain (1986). The erythrocyte indices viz Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were also calculated using the technique described by Jain (1986).

Histopathology: The selected formalinized tissues were processed after fixation and embedded in paraffin wax. The embedded tissues were then sectioned using a microtome to give 3µm thick tissue sections. The tissue sections were routinely stained with haematoxylin and eosin (H&E) before being examined under the light microscope (Olympus, Japan) for assessment of the histopathological changes (Lillie, 1965). The photomicrographs of the histopathological slides were taken using Toupview® histomorphometric camera and software.

Blood and Tissue Heavy Metal Analysis: The collected whole blood and tissues (liver and kidney) were digested using the method described by the AOAC (2004). The digested samples were then subjected to heavy metal analysis using the AAnalyst 200 Perkin Elmer Atomic Absorption Spectrophotometer (AAS) to determine the mean cadmium level for each of the samples.

Statistical analysis

The haematological parameters were expressed as Means ± SD. The blood, kidney and liver heavy metal concentration and haematological parameters were analyzed by analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences version 20.0). Statistical significance was assumed at the p - value of $p < 0.05$.

RESULTS

Cadmium Level

Blood: 43 (86 %) of the examined frogs had blood cadmium levels above the maximum FAO permissible limit (0.5mg/kg) and constituted the Above Permissible Limit (APL) group while the remaining 7 (14%) constituted the Below Permissible Limit (BPL) group. The highest blood cadmium level observed in this study was 2.28 mg/kg while the lowest observed was 0.1 mg/kg (Table 1). As shown in figure 1. the blood cadmium level (0.96±0.46 mg/kg) was the lowest compared to the levels reported in the kidney and the liver. In this study, all the frogs with blood cadmium level below the permissible limits were female.

Kidney and Liver: The liver and kidney cadmium levels in all the sampled frogs presented high values which were higher than the FAO permissible limit in both tissues. The range of the kidney cadmium level was 0.8 – 3.36 mg/kg while the liver cadmium level range was from 1.44 – 6.4 mg/kg. As seen in figure 1, the Mean±SD tissue cadmium level is highest in the liver (3.02±1.23 mg/kg) followed by the kidney (1.93±0.68 mg/kg). The Mean±SD tissue cadmium levels in the male and female frogs is also presented in table 1 and shows a comparatively higher blood, liver and kidney cadmium level in the female frogs.

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Haematology

As shown in the table 2 there was a significant difference in the haematological parameters between the frog in the BPL group and those in the APL group. The erythrocyte parameters (PCV, Hb and RBC) and the platelet count were significantly higher in the BPL group than the APL frog group. The leucocyte parameters (white blood cell count, heterophil, lymphocyte, basophils and eosinophils) were higher in the frogs in the APL group while the monocyte count was higher in the frogs in the BPL group.

Histopathology

Histopathological examination of the liver tissue revealed marked loss of the normal hepatic architecture and diffuse hepatocellular degeneration. There was also a marked accentuation of the melano-macrophage centre in the liver (Figure 3). In the kidney (Figure 4), there was a mild degeneration of the renal tubules along with mononuclear infiltration. There was also a mild increase in the glomerular cellularity along with obliteration of the bowman space in a few glomeruli and mild glomerular capsule congestion. There was a characteristic denudation of the seminiferous tubules with loss of the germ cells and exposure of the Sertoli cells and the basement membrane (Figure 5).

Table 1. Means ± SD of the blood, kidney and liver cadmium level in male and female crowned bullfrog (*Hoplobatrachus occipitalis*) captured in Ibadan, Oyo State, Nigeria

Sex (n = 50)	Mean Cadmium Level (mg/kg)		
	Blood	Liver	Kidney
Male	0.94±0.39	3.00±1.27	1.54±0.41
Female	0.98±0.54	3.03±1.25	2.17±0.72

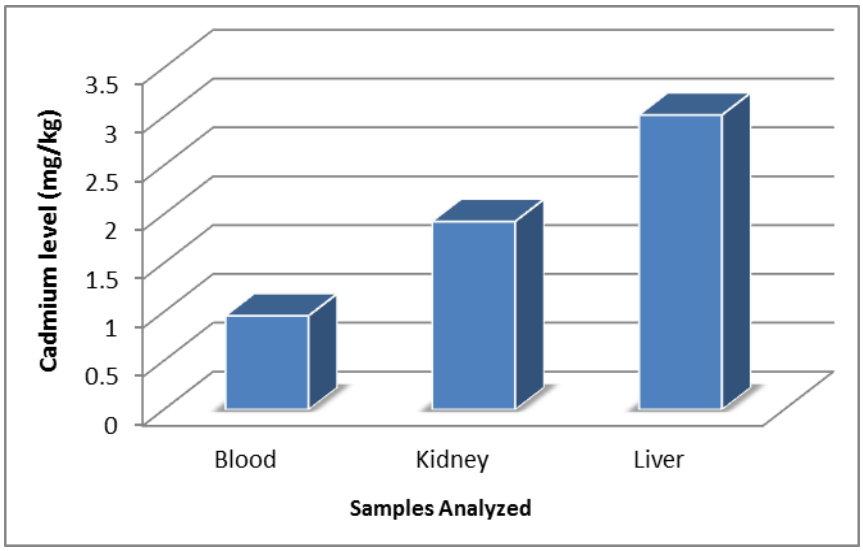

Figure 1. Mean blood, kidney and liver cadmium level in crowned bullfrog (*Hoplobatrachus occipitalis*) captured in Ibadan, Oyo State, Nigeria

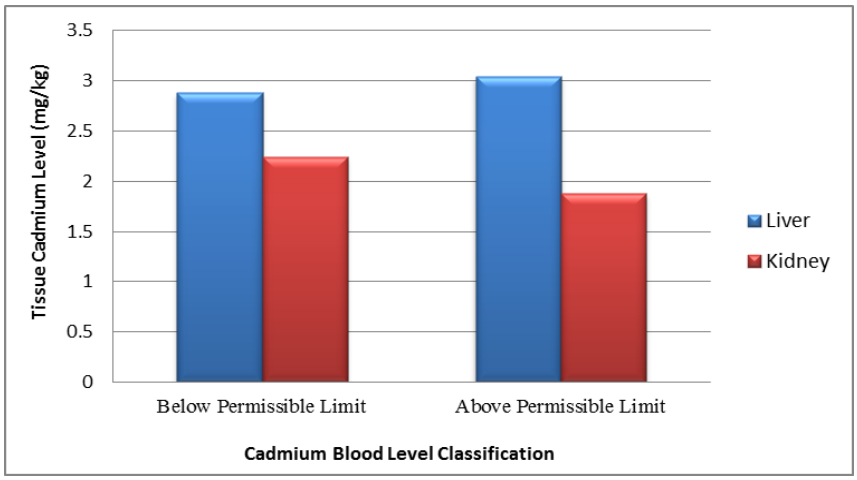

Figure 2. Mean tissue cadmium level for each blood cadmium permissible limit group in the crowned bullfrog (*Hoplobatrachus occipitalis*) captured in Ibadan, Oyo State, Nigeria

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Table 2. Means±SD of the haematological parameters of the crowned bullfrog (*Hoplobatrachus occipitalis*) captured in Ibadan, Oyo State, Nigeria with blood cadmium level permissible limit

Haematological Parameters	Cadmium Blood Level (mg/kg)	
	Below Permissible Limit n=7	Above Permissible Limit n=43
Packed Cell Volume- PCV (%)	31.57±4.61	23.00±6.60
Haemoglobin Concentration (g/dl)	8.57±2.04	7.39±2.20
Red Blood Cell ($\times10^3/\mu L$)	2.61±0.89	2.20±0.74
Mean Cell Volume (fl)	132.16±40.97	108.86±28.29
Mean Cell Haemoglobin (pg)	34.98±10.33	34.98±10.02
Mean Cell Haemoglobin Concentration (g/dL)	27.03±4.41	32.00±2.23
White Blood Cell ($\times10^3/\mu L$)	13.96±3.91	15.59±3.66
Lymphocytes ($\times10^3/\mu L$)	8.35±2.07	9.19±2.52
Heterophils($\times10^3/\mu L$)	4.61±1.72	5.18±1.56
Monocytes ($\times10^3/\mu L$)	0.51±0.36	0.47±0.29
Eosinophils ($\times10^3/\mu L$)	0.49±0.18	0.70±0.32
Basophils ($\times10^3/\mu L$)	0±0	0.05±0.08
Platelets($\times10^5/\mu L$)	1.68±0.82	1.37±0.37

FAO/WHO Permissible Limit (mg/kg) = 0.5mg/kg; n= number of samples

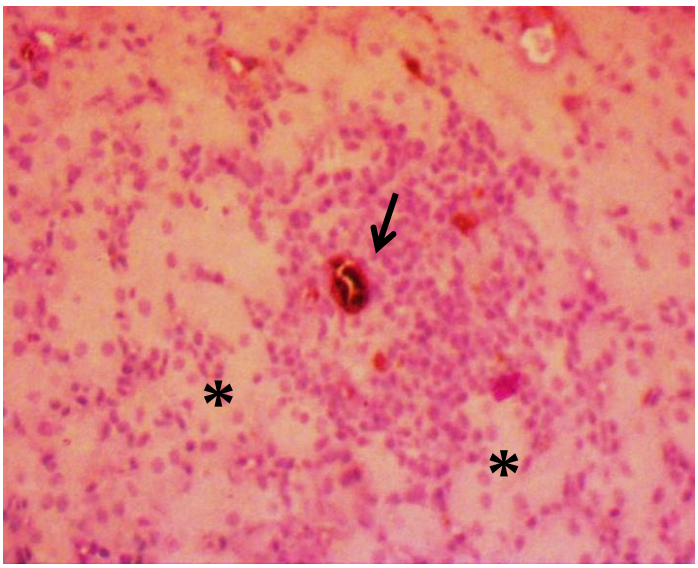

Figure 3. Liver tissue of the crowned bullfrog (*Hoplobatrachus occipitalis*) showing diffuse hepatocellular degeneration and loss of the normal hepatic architecture (asterisk). There is also a marked accentuation of the melano-macrophage centre (black arrow). ×1000 H&E

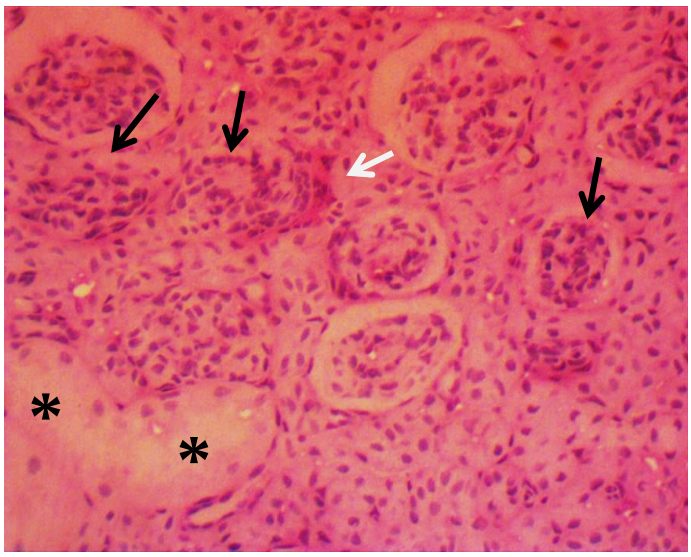

Figure 4. Kidney of the crowned bullfrog (*Hoplobatrachus occipitalis*) showing mild degeneration of the renal tubules (asterisk). There is also mild focal glomerulonephritis with increased cellularity of the glomeruli along with obliteration of the Bowman space (black arrows) in a few glomeruli. There is also a mild glomerular capsule congestion (white arrow). ×1000 H&E

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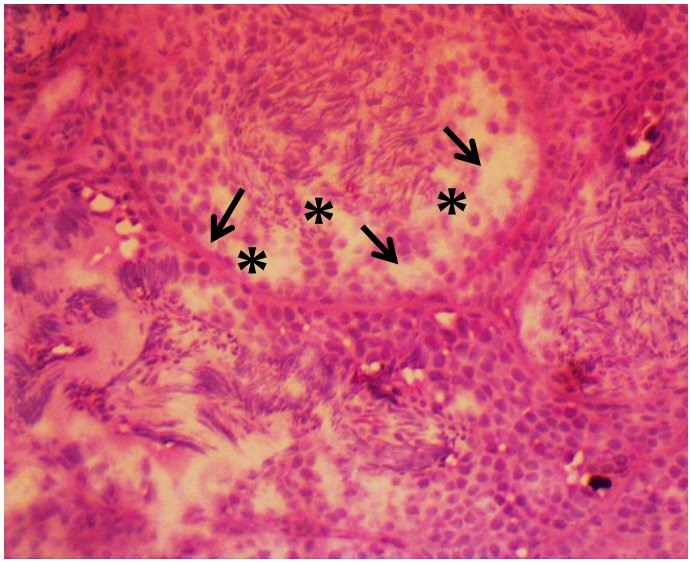

Figure 5. Testes of the crowned bullfrog (*Hoplobatrachus occipitalis*) showing loss of germ cells (asterisks) with exposure of the Sertoli cells and the basement membrane (black arrow). ×1000 H&E

DISCUSSION

Heavy metals such as cadmium, lead, copper and zinc are regarded as serious pollutants because of their toxicity and tendency to persist in the environment and be incorporated into the food chain (Kishe and Machiwa, 2003). Cadmium has been reported by different authorities as an important heavy metal pollutant in the environment with considerable ecotoxicological impact (International Programme on Chemical Safety (IPCS), 1992; International Agency for Research on Cancer (IARC), 1993 and Agency for Toxic Substances and Disease (ATSDR), 2012). Aquatic and amphibious organisms have been reported as perfect sentinel animals with the ability to accumulate heavy metal from various sources including soil, water bodies, erosion and waste water/effluents (Francis et al., 1984). With the spate of the increased discharge of cadmium in the environment, the use of frogs in this study is crucial as it helps in the assessment of the ecotoxicological impact of cadmium toxicity on the frog population. This is important due to the amphibious nature of frogs (exposing them to cadmium pollution both in the aquatic and terrestrial habitat) making them both an excellent sentinels for environmental toxicology and for the appraisal of the impact of cadmium toxicity on global frog population decline.

As shown in this study, there was an 86% prevalence of elevated blood cadmium level above the permissible limit (0.5mg/kg) in the sampled frogs. This observed high prevalence and elevated blood cadmium level corresponds to reports by other authors in which similar elevated blood cadmium levels were observed in frogs found in polluted environments and exposed to toxic environmental cadmium level (Loumbourdis et al., 2007 and Othman et al., 2009).

The tissue cadmium levels however were observed to be above the permissible limit in all the observed frogs in this study thus implying the severity of the cadmium exposure risk in the environment. The 100% prevalence of elevated tissue cadmium level observed corresponds with the similar elevated blood cadmium level observed although 14% of the sample frogs had blood cadmium level below the permissible limit. This could be associated with the tissue bioaccumulation of cadmium in frogs due to prolong exposure thus causing the tissue level to remain significantly high due to the slow release and excretion of the tissue residue over time (Agency for Toxic Substances and Disease Registry (ATSDR), 2013).

A comparatively higher cadmium level was detected in the female frogs compared to the male. This can be related to a similar finding by Vahter et al. (2007) who also reported a similar higher cadmium level in women compared to men. This observation has also been ascribed to be due to the higher intestinal dietary absorption of cadmium in women (Berglund et al., 1994; Vahter et al., 2007 and Järup et al., 2009) which according to Olsson et al. (2002) has been associated with the lower blood iron status of females.

In terms of the associated pathological changes in the frogs in the different blood cadmium permissible limit groups, the reported decrease in the erythrocyte (PCV, RBC and Hb) and platelet parameters observed can be ascribed to the previously documented decrease in haematopoiesis due to the cytotoxic effect of cadmium toxicity on haematopoietic precursor cells (Drastichová et al., 2004 and Witeska et al., 2010). More so, cadmium toxicity has been associated with haematotoxic effects on circulating blood cells thus causing accelerated destruction of the blood cells (Celik et al., 2005; 2009 and Van Den Heuvel et al., 2001). This is thus responsible for the characteristic normocytic and normochromic anaemia associated with early onset manifestation of cadmium toxicity. This anaemia can also be

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secondary to the reduction in iron absorption associated with cadmium toxicity (Campbell et al., 1984 and US - NRC, 2005).

The reported increase in the leucocyte parameters and reduction in the monocyte count in the frogs in the APL group is similar to the report by Enuneku and Ezemonye (2013) who also observed an increase in the leucocyte parameters in cadmium exposed frog *Hoplobatrachus occipitalis* and toad, *Bufo maculatus.* This finding is however in slight contrast to the report by Kondera and Witeska (2013) in which there was a general reduction in all the haematological parameters of common carp (*Cyprinus carpio L.)* exposed to cadmium. The elevation in the leucocytes could be associated with the increased demand for inflammatory response cells needed in response to the inflammation, degeneration and tissue damage caused by cadmium toxicity (Gill and Pant, 1985 and Davis et al., 2008). Furthermore, the lower cytotoxic sensitivity of the myeloid series precursor cells to cadmium toxicity compared to the erythroid series could be a likely reason why the leucopoietic process is minimally affected compared to erythropoiesis and the erythrocyte parameters (Van Den Heuvel et al. 1999 and 2001).

The highest mean cadmium tissue level in the sampled frogs was found in the liver (3.02±1.23 mg/kg) followed by the kidney (1.93±0.68 mg/kg) while the least level was observed in the blood (0.96±0.46 mg/kg). This finding is consistent with the report by Jezierska and Witeska (2006) who also reported a higher cadmium bioaccumulation in the liver than in the kidney and blood. This is in contrast with the report by Loumbourdis et al. (2007) in which the kidney was observed as the main site of bioaccumulation. This makes the liver a very important tissue for the assessment of heavy metal (especially cadmium, copper and lead) exposure since the rate of accumulation in this organ is usually proportionate to the environmental level (Jezierska and Witeska 2001 and 2006). Due to the excretory role of the kidney, the kidney tends to retain a high level of heavy metal and cadmium for a longer time. This makes the kidney an important tissue for the assessment of the tissue metal level even during remediation and depuration processes when the drop in the level of exposure is causing a drop in the metal level in other tissues (Jezierska and Witeska, 2006).

The histopathological findings in the liver, kidney and the testes were also consistent with the sequela of the cadmium toxicity mode of action via cellular membrane disruption via metallothionenin binding, oxidative stress induction due to glutathione binding and alteration of biochemical parameters. The observed histopathological changes in the liver of the frogs in the APL group were also similar to the observation by Ikechukwuu and Ajeh (2011) and Medina et al. (2016). The associated accentuation of the melano-macrophage seen in this study is consistent with the report from several authors as an important defense mechanism in response to xenobiotic exposure and increased phagocytic properties in response to inflammatory changes (Loumbourdis and Vogiatzis, 2002; Agius and Roberts, 2003 and Ribeiro et al., 2011).The renal lesions were similar to the reports by Jarup et al. (2000) in which proximal tubular cells damage and dose-dependent cadmium-induced renal pathologies were observed in cadmium exposed individuals. Åkesson et al. (2005) also reported that similar findings of both glomerular and tubular pathological changes have been associated with cadmium toxicity. The testicular pathological lesions were also consistent with the previous studies of the reproductive impact of cadmium toxicity on exposed animals and humans (Lee, 1983 and Thompson and Bannigan, 2008). All the histopathological lesions in this study were consistent with previous cadmium toxicity studies and further buttress the fact that the level of cadmium exposure and tissue bioaccumulation in the sample frogs was high enough to cause the observed pathological changes.

CONCLUSION

The study ascertained the bioaccumulation of cadmium in frogs, which is associated with deleterious effects on their tissues (blood, liver, kidney and testis) and ultimately could be responsible for the population decline of the species due to poor health and reproductive defects. It has also been further established that heavy metal toxicity is still a reality in our environment as observed in this bio-indicator species. Further research should still be carried out on the effect of exposure to this dangerous metal both on the ecosystem, human health and other wildlife species for a better understanding of the deleterious level. More so, there is a need for a more responsible utilization of cadmium and the safe disposal of cadmium containing products in order to reduce the release into the ecosystem and the impact on public health.

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Competing interests

The authors declare that there are no competing interest that might have influenced the presentation and the findings of this present study.

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Isolation and Identification of *Brucella* Species from Dairy Cattle by Biochemical Tests: The First Report from Ethiopia

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ABSTRACT

Isolation of *Brucella* organism is considered as the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant for control of brucellosis using vaccination. Serological studies revealed that brucellosis is endemic in bovines in Ethiopia. Even though seroprevalence of brucellosis is established in different species of animals, so far there was no successful attempt to isolate and identify *Brucella* spp. in dairy cattle at farm level in the country. Therefore, the endeavor of the present study was to isolate *Brucella* spp. from seropositive cattle with a history of abortion. A total of 570 dairy cattle from 35 herds were screened serologically by Rose Bengal plate test based on the history of abortion in the farm. Among the tested samples 13 (2.28%) were found positive by Rose Bengal plate test screening while 33 samples were found sero negative upon serological screening test but were collected from the cattle with history of recent abortion. Forty six clinical samples were cultured which were both from *Brucella* seropositive and seronegative (dairy cattle with history of abortion) upon Rose Bengal plate test screening. Three (6.52%) samples were *Brucella* culture positive and further characterization of all the three isolates based on biochemical tests result confirmed that the pathogen was *Brucella abortus*. *Brucella abortus* was isolated from placental cotyledon 1/9 (11.1%) and vaginal swab 2/23 (8.69%) while no isolate was obtained from milk and fetal abomasal contents (abomasal aspirate) of aborted fetus. Our finding revealed the occurrence of *B. abortus* in dairy cattle of Ethiopia through isolation of the organism for the first time from seropositive dairy cattle with a history of abortion. The organisms were isolated from placental cotyledon (one isolate) and vaginal swab (two isolates) while no isolate was obtained from milk and fetal abomasal contents (abomasal aspirate) of the aborted fetus. Hence, the bacteriological isolation and identification of *Brucella abortus* from dairy cattle indicates the importance of brucellosis in dairy cattle industry of the area and potential public health implication for human population in the study areas.

Key words: Isolation, Dairy cattle, *Brucella abortus*, Biochemical test, Ethiopia

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INTRODUCTION

Brucellosis is endemic in many developing countries and is caused by *Brucella* species that affect man, domestic and some wild animals, and marine mammals (Bhatia and Narain, 2010; Seleem et al., 2010 and Geresu et al., 2016). An estimated 500 000 new human *Brucella* cases were reported annually worldwide (Pappas et al., 2006). Brucellosis is the second most important zoonosis after rabies and has gained prominence over the years since its discovery on the island of Malta (Seleem et al., 2010 and Abubakar et al., 2012).

Brucellae species are gram negative cocci bacilli, which are classified into species by various techniques such as growth patterns on media and phage susceptibility. There are six "classical" recognized species; *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* (Godfroid et al., 2005; Hadush and Pal, 2013). Recently, four new *Brucella* species have been recognized and classified, namely, *B. pinnipedialis*, *B. ceti*, *B. microti* and *B. inopinata* (Foster et al., 2007; Scholz et al., 2009).

The mode of transmission of the bacteria varies with the epidemiological area, the animal reservoir and the occupational exposed groups (Seleem et al., 2010 and Geresu et al., 2016). Sources of infection for the transmission of the bovine brucellosis are aborted fetuses, the fetal membranes after birth, and vaginal discharges and milk from infected animals (Tolosa et al., 2010 and Geresu et al., 2016). The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water, and after birth, fetuses, and newborn calves, all of

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which may contain a large number of the organisms and constitute a very important source of infection. The bacteria can be transmitted to humans through direct contact with infected tissue via breaks in skin, ingestion of contaminated tissues or milk products, and inhalation or mucosal exposure to aerosolized bacteria (Radostits et al., 2007).

The diagnosis of brucellosis is based on serological, bacteriological, allergic skin reaction, and molecular methods (Simsek et al., 2004). The most important confirmatory method of *Brucella* infection is bacteriological diagnosis since its specificity is much higher than that of other diagnostic methods and it is used as a gold standard diagnostic method. The existence of different *Brucella* biotypes among the *Brucella* spp. and their identification is important to confirm the infection and trace the source of the infection (Guler et al., 2003). Because of the complications involved in the diagnosis of the disease, including the difficulties in distinguishing between infected and vaccinated animals by conventional serological tests, bacteriological isolation and identification of biotypes of the etiological agent are necessary steps in the design of epidemiological and eradication programs (Refai, 2002; Zinstag et al., 2005). Molecular diagnostic methods are also currently being used for the detection of *Brucella* spp. in various samples (Şahin et al., 2008).

Samples for *Brucella* spp. isolation from cattle include fetal membranes, particularly the placental cotyledons where the number of organisms tends to be very high. In addition, fetal organs such as the lungs, bronchial lymph nodes, spleen and liver, as well as fetal gastric contents, milk, vaginal secretions and semen are samples of choice for isolation (Poester et al., 2006 and Lage et al., 2008). Vaginal secretions should be sampled after abortion or parturition, preferably using a swab with transporter medium, allowing isolation of the organism up to six weeks post parturition or abortion (Poester et al., 2010). Milk samples should be a pool from all four mammary glands. Non pasteurized dairy products can also be sampled for isolation (Lage et al., 2008; Poester et al., 2010).

Since the first report of brucellosis in the 1970s in Ethiopia, the disease has been noted as one of the important livestock diseases in the country (Ibrahim et al., 2010; Kebede et al., 2008; Geresu et al., 2016). A large number of studies on bovine have been reporting individual brucellosis seroprevalence ranging from 1.1% to 22.6% in intensive livestock management systems (Tolosa et al., 2010; Tesfaye et al., 2011) and 0.05% -15.2% in extensive management systems (Degefa et al., 2011; Megersa et al., 2011).

Though serological survey for *Brucella* antibodies revealed that brucellosis is known to be endemic in the country, there was no successful attempt to isolate and identify *Brucella* spp. in dairy cattle at farm level. Therefore, the present study aimed to isolate *Brucella* spp. from dairy cattle with a history of abortion for the first time in Ethiopia by using standard cultural methods in order to establish an epidemiological base for studies on the control and prevention of brucellosis in the country.

MATERIALS AND METHODS

Study areas and design

The study was conducted in two purposely selected sites in central Ethiopia, Bishoftu, East Shewa zone and Assela, East Arsi zone. These study areas were selected based on the abundance of dairy farms that constituted the known milk sheds (Land O'Lakes Inc, 2010; Geresu et al., 2016). Bishoftu is located at 47kms south east of Addis Ababa. The area is located at 9°N latitude and 40°E longitudes at an altitude of 1850 meters above sea level in the central high land of Ethiopia. It has an annual rainfall of 866mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26°C and 14°C respectively, with mean relatively humidity of 61.3% (ADARDO, 2007). Farmers in the vicinity of Bishoftu town use a mixed crop and livestock farming system. Moreover, Bishoftu and its surrounding have variable and yet representative agroecologies of the country. These agroclimatic zones are inhabited with different plant and animal species (Conway and McKenzie, 2007).

The second study area was Asella, which is located at 175 km south east of Addis Ababa and the altitude and annual rainfall of the area ranges from 502-4130 meters above sea level and 200-400mm, respectively with mean annual temperature of 22.5^{o}C. It is one of the highly populated area in Ethiopia with estimated human population of 2521349 and livestock population of cattle, 82190; sheep, 51292; goat, 811479; poultry, 562915 and equine, 22055 (Deselegn and Gangwar, 2011; Geresu et al ., 2016).

Definitions

In Ethiopia dairy cattle production systems are classified into rural smallholder (mixed crop-livestock) production, pastoral and agro pastoral production, urban and peri-urban smallholder dairy production, and commercial dairy production systems (Land O'Lakes Inc, 2010; Asmare et al., 2013). This study focuses on the latter two production systems.

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Study population

The target populations were dairy cattle in urban and peri-urban dairy (both smallholder and commercial) farms of Asella and Bishoftu towns which are composed of Holstein Friesian, their crosses and local breeds established in the major milk sheds of the study sites (Asmare et al., 2013; Geresu et al., 2016).

Study design and sample size determination

A cross-sectional study design was conducted to isolate *Brucella* spp. infecting dairy cattle by tracing back RBPT sero screening and abortion history of the animals. Dairy cattle above six months of age were selected for this study. The sampling was performed using a two level approach, selecting first individual farms with abortion history and then randomly selecting individual animals systematically inside each farm, while all animals in each farm with recent history of abortion were sampled purposely for bacteriological culture and isolation. A list of dairy farm was prepared for each of the two study areas in collaboration with the respective district livestock health departments.

The sample size for sero screening of cattle in Asella was calculated on the basis of previous report of 14.14% sero prevalence of bovine brucellosis in Arsi Zone (Deselegn and Gangwar, 2011). Therefore to determine the sample size of dairy cattle in this area, 14.14% was used as pexp and 95% confidence interval and 5% required precision (Thrusfield, 2007).

$$n = \frac{1.96^2 \times P_{exp} x 1\text{-}P_{exp})}{d^2}$$

$$n = \frac{3.84 x .1414\ (1\text{-}0.1414)}{(0.05)^2} = 186$$

In Bishoftu, since there was no previous study done in the area, by considering 50% expected prevalence, 95% confidence interval and 5% required precision, 384 cattle were selected for this study (Thrusfield, 2007). Hence, a total of 570 dairy cattles (186 from Asella and 384 from Bishoftu) were considered for this study from 35 farms in the study areas.

Bacteriological sample collection

Fetal and placental cotyledon: Aborted cattle fetuses (aspirate of stomach content) and placental cotyledon were collected during the visits to the farms after the report of bovine abortion cases and transported to National Animal Health and Diagnostic Center (NAHDIC) in ice packs and stored at -20 ˚C until processed.

Milk: Initially the cattle in the dairy farm were screened serologically using RBPT and positive animal's milk samples were collected. Samples of milk were collected cleanly after washing and drying the whole udder and disinfecting the teats. The samples were containing milk from all quarters, and 10 ml of milk was taken from each teat. The first streams were discarded and the sample was milked directly into a sterile vessel and transported to NAHDIC in ice packs and stored at +4 ˚C until processed.

Vaginal discharge: A vaginal swab was taken after abortion or parturition in Stuart medium and transported to NAHDIC in ice packs and stored at -20 ˚C until processed.

Bacteriological isolation of *Brucella*

Brucella isolation from fetal and placental cotyledon was performed according to Farrell method (Farrell, 1974). Approximately 1ml of fetal abomasal contents and placental cotyledon collected were rubbed on to *Brucella* medium base supplemented with 5% horse serum (Oxoid, CM 0169) and onto Farrell's medium, selective medium, which is prepared by the addition of *Brucella* selective supplement (Oxoid,SR0083A) containing [polymyxin B (as SO4) = 2,500IU, bacitracin =12,500IU, cycloheximide = 50.0mg, nalidixic acid = 2.5 mg, nystatin =50,000 IU, vancomycin (as HCL) = 10.0 mg)], 5% horse serum , 50% methanol and 50% dextrose on *Brucella* medium base and tryptic soy agar. Milk samples for isolation of *Brucella* were processed according to Tantillo et al. (2003).

The milk samples were centrifuged at 3000 rpm for 10 minutes to obtain the sediment-cream mixture which then was cultured on both basal media (*Brucella* medium base supplemented with 5% horse serum) and Farrell's medium (*Brucella* selective medium). Vaginal swabs were streaked on to solid media similar to that of above mentioned clinical specimen and incubated.

The inoculated plates from different clinical specimen were incubated at 37ºC both in the absence and presence of 10% CO_2 for up to 2 weeks. After the incubation, the suspected colonies were examined for *Brucella* spp. growth. *Brucella* suspected colonies were characterized by their typical round, glistening, pinpoint and honey drop-like appearance and examined for Gram stain and modified Ziehl-Nelsen stain (MZN) initially. Subsequent biochemical tests for oxidase, catalase, urease production, methyl red, voges proskauer test, acid production on media containing

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glucose, citrate utilization, indole test, motility (at both 37°C and 20°C) were carried out. Absence of growth on MacConkey agar and non-hemolytic appearance on blood agar were also conceded.

RESULTS

Among 570 tested samples, 13(2.28%) were found positive by RBPT. The higher sero screening result was observed in and around Asella town (5.38%) compared to Bishoftu (0.78%). Thirty three different samples were included from dairy cattle with a history of recent abortion but were seronegative upon sero screening by RBPT (Table 1). Of 46 clinical samples cultured in the present study, an overall rate of 6.52% (3/46) isolation was found. All the three isolates were obtained from the RBPT positive samples while no isolate was obtained from dairy cattle with a history of abortion which are sero negative upon RBPT screening. All the three isolates were from Asella while no isolate was obtained from Bishoftu.

The result of biochemical tests revealed that all the three isolates were *B. abortus*. *Brucella abortus* was isolated from placental cotyledon 1/9 (11.1%) and vaginal swab 2/23 (8.69%) while no isolate was obtained from milk and fetal abomasal contents (abomasal aspirate) of dairy cattle (Table 2). Three colonies of *B. abortus* were observed on *Brucella* selective media (Farrell's medium) after 4 days of incubation, as pinpoint, round, convex with smooth margin, translucent and pale honey in color (Figure 1). All the colonies were grown in 10% CO_2 supplied incubator and agglutinated with positive control serum for *B. abortus* while negative with the negative control serum (slide agglutination test with an anti-*Brucella* polyclonal serum). The culture smear showed Gram negative coccobacilli in Gram's staining (Figure 2) and red stained coccobacilli in modified Ziehl-Neelsen staining (Figure 3). The isolated colonies were not grown on MacConkey agar (non-lactose fermenter) (Figure 4) and non hemolytic on blood agar (Figure 5). Growth was noticed in plate with basic fuchsin (Figure 6). The detailed result of basic biochemical and metabolic profiles of field *B.abortus* isolated from the study area were depicted in Table 3.

Table 1. Culture results of RBPT seropositive (aborted or not) and samples from dairy with abortion history (RBPT seronegative) from Asella and Bishoftu towns in 2014

Origin	Total sample	RBPT vs. Culture			Samples from dairy cattle with abortion history (RBPT seronegative)		
		RBPT +ve	Culture		N	Culture	
			+ve	-ve		+ve	-ve
Bishoftu	384	3(0.78)	0	0	14	0	14
Asella	186	10(5.38)	3	7	19	0	19
Total	570	13(2.28)	3	7	33	0	33

N = number of samples cultured, +ve = positive, -ve = negative, RBPT = Rose Bengal plate test, vs. = versus

Table 2. *Brucella* isolates recovered from clinical samples of seropositive animals from Asella dairy farm in 2014

Sample type	No. of examined samples	+ve culture isolation	%
Foetal abomasal content	1	0	0
Placental cotyledon	9	1	11.11
Milk	13	0	0
Vaginal swab	23	2	8.69
Total	46	3	6.52

No = number, +ve = Positive, % = Percent

Table 3. Basic biochemical and metabolic profiles of field *B. abortus* isolated from cattle of Asella dairy farm in 2104

Brucella isolate	Biochemical properties										Growth on dyes
	Cat	Oxi	[a]Ure	Mot	Ind	MZN	Cit	MR	VP	Glu	BF
Placental cotyledon	+	+	+	-	-	+	-	-	-	-	+
Vaginal swab 1	+	+	+	-	-	+	-	-	-	-	+
Vaginal swab 2	+	+	+	-	-	+	-	-	-	-	+

Cat = Catalase, Oxi = Oxidase, Ure = Urea hydrolysis, Mot = Motility test [+ , motile, - , non-motile], Ind = Indole production, MZN = Modified Zeihl Neelsen stain, Cit = Citrate Utilization; MR = Methyl Red, VP = Voges Proskauer, Glu = Glucose, BF = Basic Fuchsin, TSA = Tryptic Soy Agar, + = Positive test result, - = Negative test result; [a]Urea hydrolysis = All isolate positive within 2 hours of culture (Figure 7)

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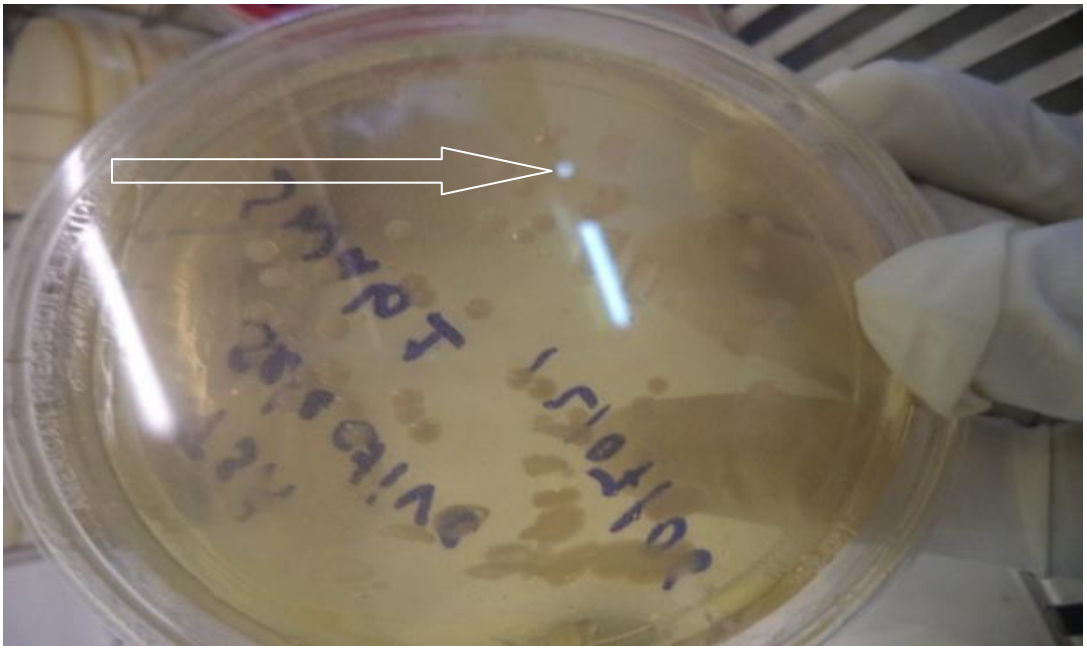

Figure 1. *Brucella* spp growth (morphology) on Farrell's medium from placental cotyledon of aborted dairy cattle in Asella town dairy farm

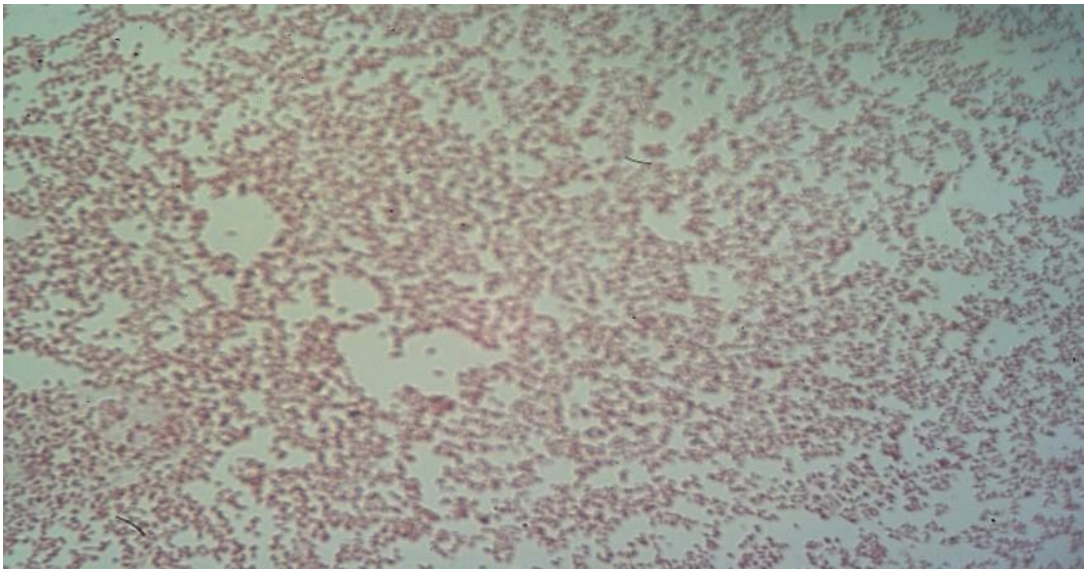

Figure 2. Gram's stain (coccobacilli) result of the isolated colony of *Brucella* ssp isolated from dairy cattle

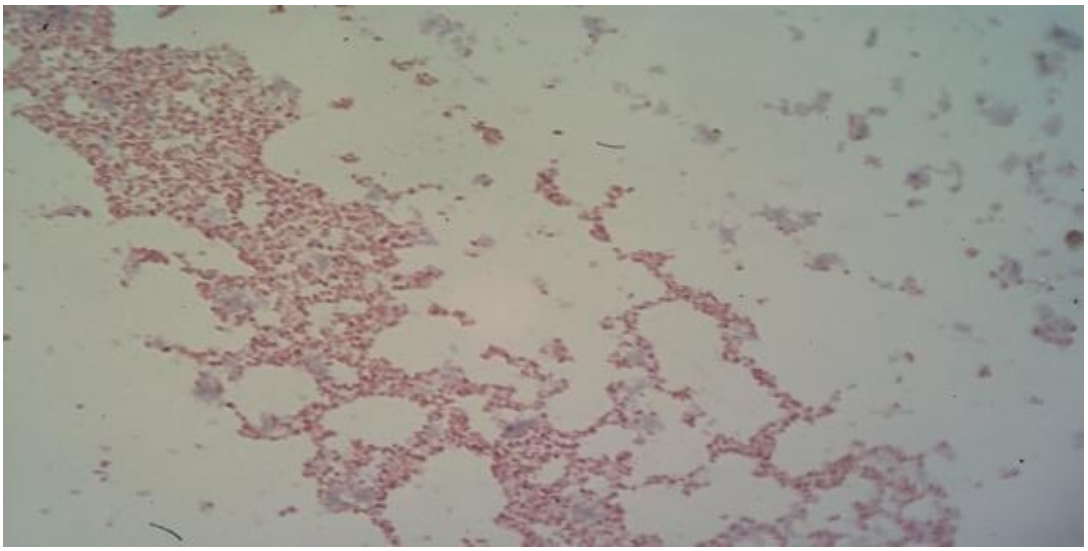

Figure 3. Modified Zeihl Neelsen (Red coccobacilli against blue back ground) stain of *Brucella* isolated from dairy cattle

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Figure 4. No growth of the isolated *Brucella* spp isolated from dairy cattle on Mac Conkey agar


Figure 5. Non-heamolytic appearance of the colony of *Brucella* spp isolated from dairy cattle on blood agar

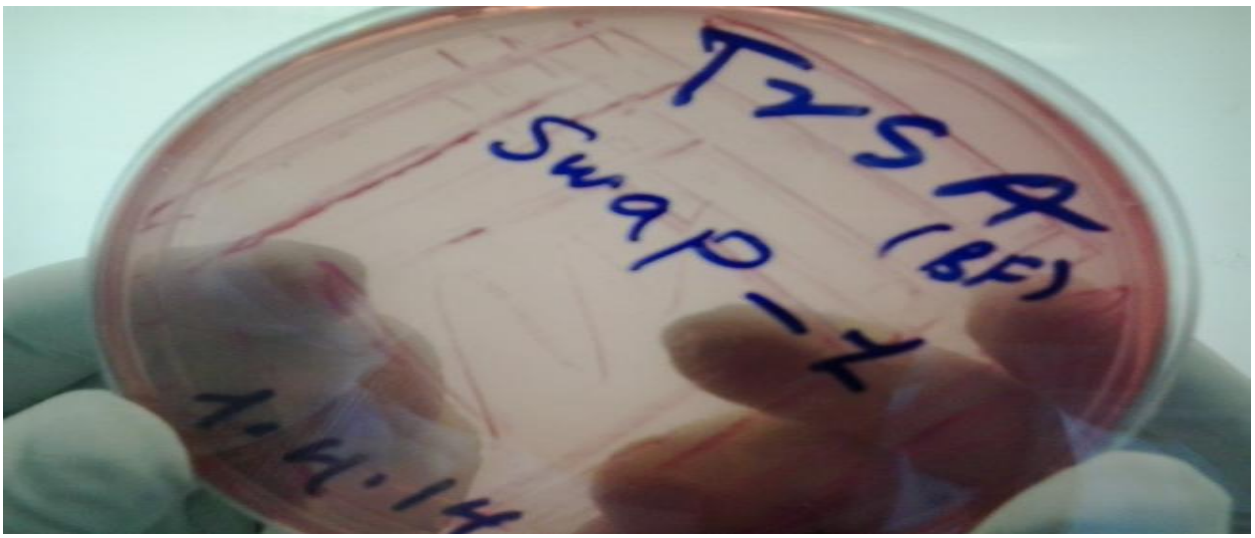

Figure 6. Growth of *Brucella* spp. isolated from dairy cattle on media containing basic fuchsin dye

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Figure 7. *Brucella* spp isolated from dairy cattle hydrolyzing urea within two hour

DISCUSSION

Sero prevalence studies in animals show that brucellosis is endemic in Ethiopia (Tolosa et al., 2010; Degefa et al., 2011; Megersa et al., 2011Tesfaye et al., 2011; Asmare et al., 2013; Geresu et al., 2106). However, the *Brucella* species and their biovars endemic in Ethiopia are unknown.

In the present study, the isolation of *B. abortus* from sero positive cattle with history of abortion was carried out in Ethiopia for the first time. This confirmatory isolation of *B. abortus* was from clinically aborted cattle placental cotyledon (11.1%) and vaginal swab (8.69%) while no isolate was obtained from milk and fetal abomasal content. The low isolation rate (6.52%) of *B. abortus* obtained in the present study from sero positive animals with a history of abortion was in agreement with previous report of 6.4 % (Çelebi and Otlu, 2011). This might be because of the slow growing and fastidious nature of the pathogen (Seleem et al., 2010).

In contrast to this result, a higher rate of isolation of *B. abortus* was reported by Gülhan et al. (2011) (26.7%), Ali et al. (2014) (40%) and Ünver et al. (2006) (55.6%) from aborted cattle fetuses. This difference may be related to the usage of more than one selective culture media in their study while in the present study only Farrell's medium was used. Isolation of *B.abortus* can be improved if more than one selective culture medium is used (Ali et al., 2014). In the present study, bacteriological cultural, morphological and biochemical tests confirmed that all the three isolates obtained from the cases of placental cotyledon and vaginal swabs of aborted cattle were *B. abortus*. Similar to the earlier reports, all the *Brucella* isolates found in this study were positive for catalase, oxidase and urea hydrolysis and negative for indole production, citrate utilization, methyl red, and Voges-Proskauer tests revealing them to be *Brucella* spp. (Koneman et al., 1997). Other reports have also indicated that on the basis of cultural, morphological, and biochemical characteristics, it is possible to identify *Brucella* spp. (Alton et al., 1988; Koneman et al., 1997).

Shedding of *Brucella* in the milk of infected animals is an important source of transmission of disease to humans if the raw milk is consumed (Tantillo et al., 2003). Ocholi et al. (2004) isolated *Brucellae* from milk (7.2 %) in Nigeria while Ali et al. (2014) recovered *B. abortus* (3.2%) from milk samples in Pakistan.

In contrary to these authors' findings, there was no recovery of *Brucella* spp. from 13 milk samples of RBPT positive cows in the present study. This result might be due to the secretion of an organism in the milk a few days (2 to 5 days) after abortion, small number of sample cultured and use of only Farrell's medium in the present study. The isolation of *Brucella* from milk samples may be improved if more than one culture medium is used (Ali et al., 2014). Hence, the result should not underestimate the risk of consuming raw milk as source of *Brucella* infection in the study area.

The fact that *Brucella* species were isolated from milk of cattle from different studies with different rate in Nigeria (Ocholi et al., 2004), Turkey (Çelebi and Otlu, 2011) and Pakistan (Ali et al., 2014) illustrates the need for further investigation in Ethiopia.

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CONCLUSION

Bovine brucellosis caused by *B.abortus* has a major impact on human health, besides causing significant economic losses in dairy industry. In Ethiopia, despite of a number of research reports on sero prevalence of brucellosis in cattle and widespread occurrence of brucellosis in different production system, there is no bacteriological isolation and identification of *B.abortus* from dairy cattle. In the present study, *B.abortus* was isolated for the first time in Ethiopia from seropositive dairy cattle with history of recent abortion. The organisms were isolated from placental cotyledon (one isolate) and vaginal swab (two isolates). Hence, it is of practical importance to isolate *Brucella* spp. to design and utilize effective *Brucella* vaccines in Ethiopia.

Acknowledgments

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Competing interests

The authors declare that they have no competing interests.

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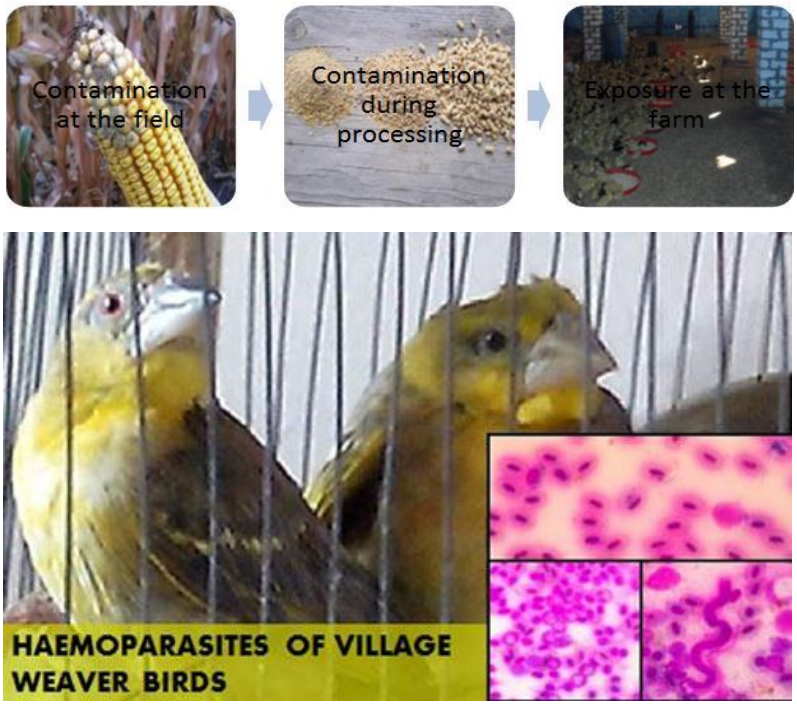

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Milligram	mg	**hours**	h
Micrometer	mm	**Minutes**	min
Molar	mol/L	**Mililitre**	ml
Percent	%		

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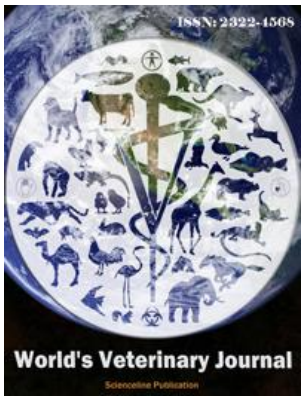

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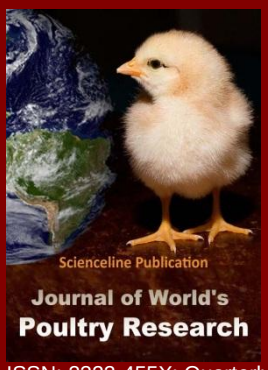

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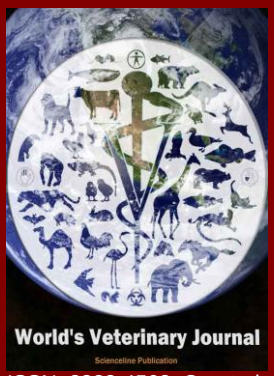

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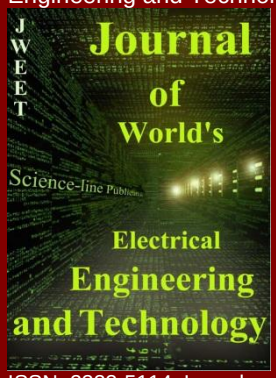

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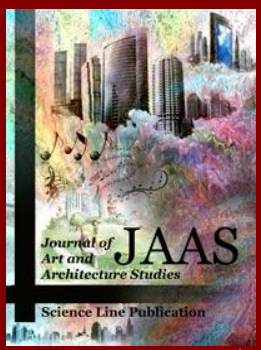

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