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## TARGET PRIORITY TRANSBOUNDARY ANIMAL DISEASES AND ZONOSSES IN THE IGAD REGION FOR CONTROL INTERVENTIONS

Magona J W<sup>1,2</sup>, Kwai A M<sup>1</sup>, Olaho-Mukani W<sup>2</sup>, Aziz A A<sup>2</sup>, Boussini H<sup>2</sup>, Wakhusama S W<sup>1,2</sup>, Muriuki S<sup>2</sup>, Zelalem T<sup>2</sup>, Wamalwa W K<sup>1</sup>, Ameha S<sup>1</sup>, Munyua S J M<sup>1</sup>

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### Abstract

Prioritization of transboundary animal diseases (TADs) and zoonoses in the IGAD region was conducted by IGAD Member States, namely, Djibouti, Ethiopia, Eritrea, Somalia, South Sudan and Sudan together with support from ICPALD and AU-IBAR. The main aim of the exercise was to develop clear control strategies and policies for focussing disease control efforts and the limited resources on few diseases in order to achieve efficient and effective disease control. The prioritization methodology for transboundary animal diseases and zoonoses according to Phylum was applied, taking into considerations, economic impact, human health importance, societal impact, environmental impact, feasibility of control methods and the ecological impact of the diseases. Overall, *Rift Valley fever* had the highest rank, followed by *Contagious Bovine Pleuropneumonia*, *Newcastle Disease*, *Highly Pathogenic Avian Influenza*, *Lumpy Skin Disease*, *Peste des Petits des Ruminants*, *Rabies*, *Brucellosis*, *Bovine Tuberculosis*, *Foot-and Mouth Disease* and *Sheep and Goat Pox*. In conclusion, the IGAD region is encouraged to facilitate systematic efforts towards rolling forth priority diseases through focused national efforts, harmonized regional strategies and streamlined stakeholder investment in the region.

**Key words:** Target, priority, animal diseases, zoonoses, control, interventions, IGAD region

## MALADIES ANIMALES TRANSFRONTALIÈRES ET ZONOSSES PRIORITAIRES CIBLÉES DANS LA RÉGION IGAD POUR LES INTERVENTIONS DE CONTRÔLE

### Resume

Les États membres de l'IGAD, à savoir Djibouti, l'Éthiopie, l'Érythrée, la Somalie, le Soudan du Sud et le Soudan, ont procédé à une définition des maladies animales transfrontières et zoonoses prioritaires dans la région IGAD, avec le soutien de l'ICPALD et de l'UA-BIRA. L'objectif principal de cette priorisation était d'élaborer des stratégies et politiques de contrôle claires visant à concentrer les efforts de contrôle des maladies et les rares ressources disponibles sur certaines maladies afin de parvenir à un contrôle efficace et efficient. La méthodologie de priorisation des maladies animales transfrontalières et des zoonoses selon leur phylum a été appliquée, en prenant en considérations l'impact économique, l'importance pour la santé humaine, l'impact sociétal, l'impact environnemental, la faisabilité des méthodes de contrôle et l'impact écologique des maladies. Dans l'ensemble, la *fièvre de la vallée du Rift* a été mise en tête des priorités, suivie de la *péripleurmonie contagieuse bovine*, la maladie de *Newcastle*, l'*influenza aviaire* hautement pathogène, la dermatose nodulaire contagieuse, la *peste des petits ruminants*, la *rage*, la *brucellose*, la *tuberculose bovine*, la fièvre aphteuse et la clavelée du mouton et la variole caprine. En conclusion, la région IGAD est encouragée à faciliter des efforts systématiques visant à contrôler les maladies prioritaires à travers des activités nationales ciblées, des stratégies régionales harmonisées et une rationalisation des investissements des parties prenantes dans la région.

**Mots-clés :** ciblées, prioritaire, maladies animales, zoonoses, contrôle, interventions, région IGAD

## Introduction

Livestock rearing is a significant livelihood and food security activity among inhabitants of the IGAD region and the Greater Horn of Africa (GhoA). The livestock population in the region is estimated at 130 million cattle, 123 million goats, 111 million sheep, 900 million poultry, 18 million camels and 2.7 million pigs (IGAD 2014). The region is the leading livestock hub in Africa and has a livestock sector with a great and largely untapped potential that can substantially contribute to food security and general regional economic integration (IGAD 2014). Despite the enormous livestock resource, animal productivity, access to market and public health are greatly compromised by rampant animal diseases afflicting the IGAD region in particular and the Greater Horn of Africa in general. All the major transboundary animal diseases and zoonoses are present in the region, with the majority being endemic (IGAD 2014). Endemic animal diseases in the region include Foot and Mouth Disease (FMD), Newcastle Disease (ND), *Contagious Bovine Pleuropneumonia* (CBPP), *Contagious Caprine Pleuro-Pneumonia* (CCPP), *Peste des Petits Ruminants* (PPR), *Lumpy Skin Disease* (LSD), *Rabies*, *Brucellosis*, *Bovine Tuberculosis*, *Sheep and Goat Pox* (Waret-Szkuta et al, 2008; OIE 2008). The livestock sector in the region suffers from enormous losses as a result of the negative impact of the endemic transboundary animal diseases.

For example, two bans provoked by *Rift Valley fever* (RVF), cost the region an estimated US\$109 million from February 1998 to May 1999 and US\$326 million from September 2000 to December 2002 (Bradbury, 2008; FSNAU, 2010; Massimo et al, 2012). The export bans impacted negatively on pastoralists' incomes due to the reduced number and value of exported animals. Reduced livestock prices and the attendant poor terms of trade further undermined pastoralists' purchasing power and weakened their resilience. Also affected were other actors employed along the livestock marketing and export chains and those providing support services to the industry. The

recurrence of RVF in Eastern Africa between 2006 and 2007 resulted in the deaths of more than 100 people in Kenya and caused significant economic losses due to animal mortalities (Munyua et al., 2010). Coupled with this, a ban was slammed on livestock imports from the Horn of Africa region by the main livestock trading partners.

Moreover, controlling of all these endemic diseases and protecting the region from emerging hazards, requires resources that neither Governments, nor private sector are able to mobilize, hence creating insurmountable challenges for Veterinary Services and livestock stakeholders in the region. In view of this scenario, prioritization of TADs and zoonoses in the IGAD region was conducted by IGAD Member States and the Secretariat with support from AU-IBAR. This was intended to develop clear control strategies and policies for the region as a measure to help focus disease control efforts and the limited resources on few diseases for efficient and effective disease control. This paper therefore describes the process, outcomes and desired actions to control the key priority animal diseases and zoonoses in the IGAD region.

## Materials and Methods

### *Geographical description* *IGAD region*

The IGAD region comprises 8 member states namely; Djibouti, Ethiopia, Eritrea, Kenya, Somalia, South Sudan, Sudan and Uganda (Fig 1). The countries collectively have the largest livestock population in Africa, kept in arid semi-arid lands receiving rainfall not exceeding 400mm annually. About 80% of the region's population keep livestock, making it a very important asset, especially for people living in the drier arid and semi-arid parts of the region. IGAD region has one of the highest ruminant livestock concentrations in the world and in Africa. The region is host to 8% of the cattle, 9.6% of small ruminants and 51% of the camel population in the world (FAOSTAT, 2012). The IGAD region accounts for 17.24% of the continents land area in Africa, with a percent

share of about 38.6% of the cattle, 30.6% of small ruminants and 60.8% of the camel population (FAOSTAT, 2012).

### Countries

#### Djibouti

Djibouti is a small strategic country for the IGAD region as the gateway to the Middle East market, given its close proximity and quarantine stations. Its livestock population is estimated at 40,000 cattle, 390,000 sheep, 610,000 goats and 50,000 camels largely kept under a pastoral livestock production system (FAOSTAT, 2012). Despite its small livestock population and low incidence of animal diseases, it is important to maintain a relatively good sanitary status, given a high flow of animals arriving from areas with poor sanitary status that cross the country to be exported.

#### Ethiopia

Ethiopia is the country with the highest number of animals in the IGAD region. Its livestock population is estimated at 53 million cattle, 24 million sheep, 22 million goats and 3 million camels (FAOSTAT, 2012). Livestock is a key factor for development of the country that justifies an ambitious animal health policy to improve the productivity of the herds. Most of the livestock systems in Ethiopia involve pastoral movement of animals to other countries for pasture and water or for export trade in live animals.

#### Eritrea

Eritrea is estimated to have 2.3 million cattle, 2.5 million sheep and 5.5 million goats (FAOSTAT, 2012). Approximately 50% of the livestock population is found in the western



Figure 1: Map of IGAD countries

lowlands, especially cattle, followed by the highlands and then the eastern lowlands.

### Kenya

The comprehensive livestock census done in 2009 established Kenya's animal resource base to be 17.5 million cattle, 27.7 million goats, 17 million sheep, 3 million camels, 31.8 million domestic birds, 1.8 million donkeys, and an undetermined number of companion, game and aquatic animals. Animal resources provide livelihoods and wealth for a majority of Kenyans and significantly contribute to the national economy. In totality, the animal resource industry contributes 16 percent of the GDP (Ministry of Agriculture, Livestock and Fisheries, 2015).

### Somalia

Somalia is estimated to have 4.8 million cattle, 12.3 million sheep, 19 million goats and 7 million camels (FAOSTAT, 2012). Livestock is by and large kept under a pastoral livestock production system. A sizeable number of livestock is exported to Middle East markets through the numerous quarantine stations (such as the ones in Berbera and Bossaso) that exist within Somalia (UN/WB, 2006; Munyua, 2008; Masake *et al.*, 2008).

### South Sudan

South Sudan has an estimated livestock population of 10 million cattle, 13 million sheep, 12 million goats and 1,000 camels (FAOSTAT, 2012). The majority of the livestock is kept under a nomadic system. Whereas livestock in South-Sudan is predominantly dedicated to the domestic market, a significant portion of the livestock normally moves to other countries due to nomadic systems.

### Sudan

Sudan is a large country with different agro-climatic zones and a large number of animals. Its livestock population is estimated at 29.8 million cattle, 39.4 million sheep, 30.8 million goats and 4.7 million camels (FAOSTAT, 2012). A large part of the livestock is raised through pastoral and agro-pastoral systems

with high livestock mobility. A large part of the production is dedicated to the Middle East market through the export of live animals.

### Uganda

Uganda has an estimated livestock population of 13.6 million cattle, 3.8 million sheep, 14 million goats, 3.6 million pigs and 44.7 million poultry (Uganda National Livestock Census Report, 2008). The livestock is kept under the pastoral, agro-pastoral and mixed-crop livestock productions systems.

### Prioritization methodology

The prioritization methodology applied for TADs and zoonoses in the IGAD region was according to PHYLUM (Phylum, 2012). The methodology involved two sequential steps in the analysis of a disease. The first step was the global characterization to assess the inherent or scientific aspects of the disease independent of any local context; and the second step was the local approach that helped assess the disease within the specific context of the country or region in question.

Global characterization involved intrinsic analysis of the disease according to available scientific knowledge. This was intended to establish the characteristics for the description of the disease profile and potential nuisance in terms of epidemiology, economic consequences and human health issues. In addition, global characterization helped create consensus among stakeholders on the fundamental data to be used in detailed analyses. It also helped in identifying possible gaps in disease knowledge. Key aspects of global characterization included presence or absence of the disease, its nature and mode of transmission. Ultimately, to obtain an overview of the availability, effectiveness and efficiency of the tools regarding control of a disease in the event of an outbreak, possible control measures of the disease were then assessed.

The local approach on the other hand was concerned with applying the global characterization to the specific context of a given country or region. A disease was then prioritized according to the specific local

context. This was because the impact of a disease in a territory was highly dependent on local perceptions, geography, production and trade systems and socio-cultural background among other factors. The local approach was a data intensive exercise requiring accurate overview of the production systems, the global importance of animal agriculture and the respective importance of the different categories of animals and animal products for the local economy. The key elements of the local assessment included; epidemiology of the disease; absence or presence of a disease; risk of introduction; impact, control strategies and their local feasibility; and impact of control measures. Other economic factors considered included the general population and indirect concerns such as human health, societal and environmental impacts.

During the diseases prioritization exercise conducted in Naivasha, Kenya for IGAD Member Countries, both sequential steps (global characterization and local approach) were performed using the Phylum tool. The disease prioritization assessment was conducted by teams from Djibouti, Eritrea, Ethiopia, Somalia, South Sudan and Sudan. Each team was comprised of five experts. The composition of the team was as follows: four veterinarians specializing in veterinary public health, laboratory, field epidemiology or wildlife health, and one public health expert dealing with zoonoses. The teams were supported by experts from ICPALD, AU-IBAR and a Consultant from Phylum. After going through the assessments, all the teams presented their findings. Thereafter, each team was allowed three weeks to discuss with other professionals and their seniors back at home before submitting their final comparative tables to AU-IBAR for final compilation.

#### Data analysis

All the information and data for the disease assessments was captured in a computerized model using an Excel Spreadsheet containing formulae and other linkages to execute computations. Data from the characterization of diseases was then

transferred to the iterative tables to allow the automated computation produce the weighted list of priority diseases. Both qualitative and quantitative elements of prioritization for the different diseases and specific criteria were generated in three categories. The categories included absent diseases, present diseases with known epidemiology, and diseases with unreliable local epidemiological knowledge as well as non-existent control strategies. The output from the tool was then subjected to a comparative and interpretative process by interdisciplinary experts and local decision makers, integrating different components of local geopolitical and socio-cultural orientation.

## Results

The findings of the prioritization exercise for the IGAD region are presented in form of weights and ranks of various diseases. Diseases are ranked according to their economic impact in Table 1. *Rift Valley fever* had the highest weight and rank, followed by Foot-and-Mouth Disease, *Highly Pathogenic Avian Influenza* (HPAI), Sheep and Goat Pox (SGP), *brucellosis*, Newcastle Disease, *Pestes des Petits des Ruminants*, *Lumpy skin Disease*, *Contagious bovine Pleuropneumonia*, *Bovine Tuberculosis* and *Rabies*.

In Table 2, diseases are ranked according to human health importance. *Rift Valley fever* had the highest rank, followed by *Rabies*, *Highly Pathogenic Avian Influenza*, *Brucellosis*, *Bovine Tuberculosis*, Newcastle Disease, Foot-and-Mouth Disease, *Pestes des Petits des Ruminants*, *Contagious Bovine Pleuropneumonia*, *Lumpy skin disease* and Sheep and Goat Pox.

Regarding societal impact, disease ranking is shown in Table 3. *Highly Pathogenic Avian Influenza* had the highest rank, followed by *brucellosis*, *Rift Valley fever*, *Contagious Bovine Pleuropneumonia*, Sheep and Goat pox, *Rabies*, *Bovine Tuberculosis*, Foot-and-Mouth Disease, Newcastle Disease, *Lumpy Skin Disease* and *Pestes des Petits des Ruminants*.

In Table 4, diseases are ranked according to environmental impact. *Bovine Tuberculosis* had the highest rank, followed by Foot-and-Mouth

**Table 1:** Ranking of diseases according to their economic impact

<b>Disease</b>	<b>Scores according to the Economic Impact</b>	<b>Rank</b>
<i>Rift Valley Fever</i>	10.000	1
<i>Foot and Mouth Disease</i>	6.304	2
<i>Highly Pathogenic Avian Influenza</i>	3.730	3
<i>Sheep and Goat Pox</i>	3.600	4
<i>Brucellosis</i>	3.555	5
<i>Newcastle Disease</i>	3.212	6
<i>Peste Des Petits Ruminantes</i>	3.115	7
<i>Lumpy skin Disease</i>	2.615	8
<i>Contagious Bovine Pleuropneumonia</i>	2.216	9
<i>Bovine Tuberculosis</i>	0.470	10

**Table 2:** Ranking of diseases according to human health importance

<b>Disease</b>	<b>Scores according to Human Health Impact</b>	<b>Rank</b>
<i>Rift Valley Fever</i>	10.000	1
<i>Rabies</i>	5.507	2
<i>Highly Pathogenic Avian Influenza</i>	5.238	3
<i>Brucellosis</i>	5.031	4
<i>Bovine Tuberculosis</i>	5.008	5
<i>Newcastle Disease</i>	5.008	5
<i>Foot and Mouth Disease</i>	1.169	6

**Table 3:** Ranking of diseases according to societal impact

<b>Disease</b>	<b>Scores according to the Societal impact</b>	<b>Rank</b>
<i>Highly Pathogenic Avian Influenza</i>	10.000	1
<i>Brucellosis</i>	8.905	2
<i>Rift Valley Fever</i>	8.000	3
<i>Contagious Bovine Pleuropneumonia</i>	4.571	4
<i>Sheep and Goat Pox</i>	4.571	4
<i>Rabies</i>	2.333	5
<i>Bovine Tuberculosis</i>	2.286	6
<i>Foot and Mouth Disease</i>	2.000	7
<i>Newcastle Disease</i>	1.143	8
<i>Lumpy Skin Disease</i>	0.286	9

**Table 4:** Ranking of diseases according to environmental impact

Disease	Scores according to the Environmental impact	Rank
<i>Bovine Tuberculosis</i>	10.000	1
<i>Foot and Mouth Disease</i>	5.000	2
<i>Peste Des Petits Ruminantes</i>	5.000	2
<i>Lumpy Skin Disease</i>	5.000	2
<i>Sheep and Goat Pox</i>	5.000	2
<i>Newcastle Disease</i>	3.333	3
<i>Rift Valley Fever</i>	2.500	4
<i>Contagious Bovine Pleuropneumonia</i>	2.500	4
<i>Brucellosis</i>	2.500	4
<i>Highly Pathogenic Avian Influenza</i>	1.667	4

**Table 5:** Ranking according to feasibility of control methods

Disease	Scores according to the feasibility of Control Measures	Rank
<i>Brucellosis</i>	10.000	1
<i>Highly Pathogenic Avian Influenza</i>	8.187	2
<i>Rift Valley Fever</i>	7.088	3
<i>Rabies</i>	6.703	4
<i>Peste Des Petits Ruminantes</i>	4.725	5
<i>Bovine Tuberculosis</i>	4.011	6
<i>Newcastle Disease</i>	3.956	7
<i>Foot and Mouth Disease</i>	3.462	8
<i>Lumpy Skin Disease</i>	3.242	9
<i>Sheep and Goat Pox</i>	1.593	10

Disease, *Pestes des Petits des Ruminantes*, *Lumpy Skin Disease*, *Sheep and Goat Pox*, *Newcastle Disease*, *Rift Valley fever*, *Contagious Bovine Pleuropneumonia*, *Brucellosis*, *Highly Pathogenic Avian Influenza* and *Rabies*.

Regarding feasibility of control methods, disease ranking is shown in Table 5. *Brucellosis* ranked highest, followed by *Highly Pathogenic Avian Influenza*, *Rift Valley fever*, *Rabies*, *Pestes des Petits des Ruminantes*, *Bovine Tuberculosis*, *Newcastle Disease*, *Foot-and-Mouth Disease*, *Lumpy Skin Disease*, *Sheep and Goat Pox* and *Contagious Bovine Pleuropneumonia*.

In Table 6, diseases are ranked according to ecological impact. *Highly Pathogenic Avian Influenza* had the highest rank followed by *Rift*

*Valley fever*, followed by *Pestes des Petits des Ruminantes*, *Contagious Bovine Pleuropneumonia*, *Newcastle Disease*, *Foot-and-Mouth Disease*, *Brucellosis*, *Sheep and Goat Pox*, *Lumpy Skin Disease*, *Rabies* and *Tuberculosis*.

Finally, Table 7 shows the overall ranking of diseases together with the justification for their ranking. *Rift Valley fever* had the highest rank, followed by *Contagious Bovine Pleuropneumonia*, *Newcastle Disease*, *Highly Pathogenic Avian Influenza*, *Lumpy Skin Disease*, *Pestes des Petits des Ruminantes*, *Rabies*, *Brucellosis*, *Bovine Tuberculosis*, *Foot-and Mouth Disease* and *Sheep and Goat Pox*.

Country-specific ranking of diseases present within each IGAD Member State was

**Table 6:** Ranking of diseases according to ecological impact

Disease	Scores according to ecological impact	Rank
<i>Brucellosis</i>	10.000	1
<i>Highly Pathogenic Avian Influenza</i>	10.000	1
<i>Rift Valley Fever</i>	9.091	2
<i>Peste Des Petits Ruminantes</i>	9.091	2
<i>Contagious Bovine Pleuropneumonia</i>	9.091	2
<i>Newcastle Disease</i>	9.091	2
<i>Foot and Mouth Disease</i>	8.182	3
<i>Brucellosis</i>	8.182	3
<i>Sheep and Goat Pox</i>	7.273	4
<i>Lumpy Skin Disease</i>	3.636	5
<i>Rabies</i>	2.727	6

**Table 7:** Overall ranking of disease considering all criteria

Diseases	Overall Scores	Rank	Justification for ranking
<i>Rift Valley Fever</i>	7.145	1	Zoonotic, high impact on trade
<i>Contagious Bovine Pleuropneumonia</i>	6.975	2	Highly contagious, affects trade and production
<i>Newcastle Disease</i>	6.644	3	High mortality in a sector of high value to the poor communities.
<i>Highly Pathogenic Avian Influenza</i>	4.445	4	perceived threat
<i>Lumpy Skin Disease</i>	4.154	5	Highly contagious, affects trade and production
<i>Peste Des Petits Ruminantes</i>	3.624	6	Highly contagious, affects trade and production
<i>Rabies</i>	3.420	7	Zoonotic
<i>Brucellosis</i>	3.371	8	Zoonotic, transmission through dairy products.
<i>Bovine Tuberculosis</i>	3.111	9	Zoonotic
<i>Foot and Mouth Disease</i>	3.102	10	Multiple major species affected, highly contagious, affects trade.
<i>Sheep and Goat Pox</i>	2.587	11	Highly contagious, affects trade and production

performed as well. In Djibouti, PPR, CCPP, CBPP, *Brucellosis*, FMD and *Bovine Tuberculosis* were identified as priorities. For Ethiopia, PPR, LSD, FMD, *Brucellosis* and *Bovine Tuberculosis* were identified as priorities. While for Eritrea, top priorities included PPR, FMD, Sheep and Goat Pox, Newcastle disease, *Brucellosis*, *Bovine tuberculosis* and *Rabies*. For Somalia, CCPP, Sheep and Goat Pox, *Brucellosis* and

FMD emerged priority diseases and for South Sudan, top priorities included FMD, PPR, RVF, *Brucellosis* and CBPP. Meanwhile, for Sudan priority diseases included FMD, PPR, Sheep and Goat Pox, CBPP, *Brucellosis* and *Rabies*.

The prioritization exercise as well dealt with diseases considered absent but paused great risk for countries in the IGAD region. For Djibouti, FMD (serotypes absent) and

HPAI were the top priorities in this category. For FMD absent serotypes, it was important to avoid introduction of new serotypes and for early detection and emergency response in case of introduction. Despite the fact that poultry is not well developed in Djibouti, HPAI was considered as a global issue requiring control in border areas and requiring capacity for early detection and emergency response.

For Ethiopia, priority absent diseases included, RVF, FMD exotic serotypes and HPAI. RVF was a top priority considering the presence of the virus in neighboring countries. For FMD exotic serotypes, prevention of introduction of new serotypes through control in border areas was important and quite a sensitive issue, given the large population of livestock in Ethiopia and free and frequent movements into neighbouring countries. HPAI was also a priority recognizing its potential impact on human health and on the poultry sector.

FMD exotic serotypes, CBPP, RVF and HPAI were considered important absent diseases for Eritrea. FMD serotype C had to be identified in order to be controlled in the same time that vaccination was developed for the circulating serotypes. CBPP had to be confirmed as an absent disease to create an added value for the country. RVF was presumed absent and had to be confirmed. Likewise, HPAI was considered an important exotic disease.

RVF, FMD (exotic serotypes) and HPAI were considered important absent diseases for Somalia. RVF was important because had been reported in a neighboring country. While FMD exotic strains had to be considered because they needed to be controlled as well during vaccination campaigns against circulating serotypes. HPAI had to be considered as well because of the global concern. For South Sudan as well, HPAI and FMD (absent serotypes) were considered priority absent diseases. For Sudan, HPAI, FMD (absent serotypes) and RVF were identified as priority absent diseases. This was because FMD absent serotypes had been considered absent in all neighbouring countries. For RVF and HPAI had been reported in a neighbouring country such as Egypt.

## Discussion

This paper presents outcomes of a disease prioritization exercise conducted for the IGAD region using the OIE-Phylum tool for the prioritization and categorization of animal diseases, including the zoonotic ones. It is largely believed that poor access to the right tools and technologies, weak animal health systems, weak disease control infrastructure and perennial shortages of key resources (human and financial resources), lack of public participation or social support are major factors hindering effective control of animal diseases including those transmissible to humans within the IGAD region. Moreover, the region has a very high burden of transboundary animal diseases and zoonoses with 12 out of the 15 most important animal diseases globally being endemic in the IGAD region (CGIAR, 2015). It was envisaged that clear identification of priorities, roles of stakeholders and the means required to control animal diseases, would catalyze more effective, efficient and sustainable processes for their prevention and control. This necessitated adoption of the Phylum tool. The tool came with several important functions, including identifying and ranking the most important diseases that warranted investment of scarce public resources; bringing multi-sectoral stakeholders and experts together to build consensus on the roles and responsibilities of different players; identifying the means to deal with them; identifying and deliberating potential strategies and interventions; identification of possible policies to be developed or enforced among others.

Disease prioritization considered several parameters, including the economic impact; human health importance; societal impact; environmental impact; feasibility of the disease control methods; ecological impact and a combination of all parameters.

Economic impact was linked to losses caused by the diseases in question. RVF followed by FMD were ranked highest on analysis of diseases according to economic impact. RVF affects several ruminant species leading to losses. Several outbreaks of RVF have been

reported within the IGAD region in past years: 1997–1998 (Woods *et al.*, 2002) and 2006–2007 (Munyua *et al.*, 2010), affecting both livestock and humans. This led to the ban on livestock exports to the Middle East (Bradbury, 2008; FSNAU, 2010; Massimo *et al.*, 2012). For FMD, it has been a subject of serious economic losses in the region given its speed of spread and a wide host range affected. Indeed, FMD is widely distributed in all the IGAD Member countries with serotypes A, O, Sat 1 and Sat 2 having been reported in Kenya, Somalia, Ethiopia, Sudan and Uganda. In addition, serotype C has been reported in Somalia, while Sat 3 is present in Uganda (RISP II, 2013).

The human health importance of diseases was related to their zoonotic nature and severe effects on food security. *Rift Valley fever* followed by *Rabies* ranked highest. This was largely because of their zoonotic nature. Other key zoonotic diseases included HPAI, *Brucellosis* and *Bovine Tuberculosis*. Other diseases such as Newcastle Disease, FMD, PPR, CBPP, LSD and SGP were included because of the severe losses in terms of reduced meat and milk they cause that normally affect food security and ultimately human nutrition and health.

Available reports indicate that RVF affected over 100,000 people in Kenya with over 450 deaths were registered during the 1998–1999 RVF outbreaks (Woods *et al.*, 2002). During the RVF outbreak of 2006 to 2007 approximately 700 human RVF cases in were registered in Kenya (Munyua *et al.*, 2010). For *Rabies*, reports indicate that the *rabies* burden in Africa is estimated at 23,800 human deaths with an estimated loss of human productivity of 609,000 DALYs (WHO, 2013).

For HPAI, the IGAD region is considered to be at risk given its proximity to Egypt. From 2003 to 2016 Egypt reported a cumulative number of confirmed human cases and deaths from Avian influenza A (H5N1) of 354 and 167, respectively (WHO, 2016).

For *brucellosis*, reports indicated the highest incidence of human *brucellosis* in Africa was recorded in Algeria at 84.3 per million of population per year, and the lowest in Uganda at 0.9 per million of population per year (Pappas

*et al.*, 2006).

For zoonotic *bovine tuberculosis*, most cases are caused by *M. bovis* and cattle are the major reservoirs. An incidence of TB cases in humans caused by *M. bovis* of 2.8% has been reported in Africa, in studies carried out in 13 countries (Muller *et al.*, 2013).

Societal impact of disease ranking involved a multiplicity of disease effects as regards the relationship between cultural and social practices and occurrences of disease. HPAI followed by *Brucellosis*, RVF, CBPP, SGP and *Rabies* were ranked highest. HPAI is a good example that illustrates the relationship of cultural and social practices and the appearance of animal diseases. For instance, Southeast Asia is considered the epicentre of recent influenza outbreaks. This is linked to agricultural practices in highly populated areas. Rice fields often have standing water that attracts waterfowls. These waterfowls are natural reservoirs; potentially spreading the disease to other domestic animals (e.g. chickens, ducks, and pigs) raised outdoors (Bender *et al.*, 2006).

For *brucellosis*, traditional beliefs and practices such as drinking of raw milk and direct handling of infected animals or aborted fetuses combined with the food habits and lifestyle of pastoralists, enhance transmission of *brucellosis* to the human population and make control of *brucellosis* in these communities difficult (Smits, 2013; McDermott and Arimi, 2002). Human behavioural factors normally influence transmission of *Rift Valley fever*. Consumption of contaminated meat and milk, handling of infected tissues during necropsy or meat preparation, inhalation of viruses during opening up dead animals and lack of protection from mosquito vectors enhances transmission of RVF virus (N'gang'a *et al.*, 2016).

The spread of CBPP is largely influenced by uncontrolled movement of infected livestock, especially in situations when movement control is difficult and impractical. This often happens in cases of transhumance for pastoral livelihoods, trade, socio-cultural practices and civil strife (Wanyoike *et al.*, 2013). SGP is highly contagious, spreading by aerosol from infected animals, from contact with the

virus in the environment and from arthropod vectors, primarily biting flies. The virus can survive in poorly maintained, unhygienic sheltered environment for six months and survives drying, freezing and thawing. It usually spreads when infected stock migrate or are transported to a naïve area. Traditional but hazardous practice of inoculating sheep with a suspension of infected scab material (ovination) also leads to transmission of the virus in many countries (Watt and Serveuer, 2014).

For *Rabies*, domestic dogs are considered to be the main source (>90%) for human *rabies* in Africa. Practices such as poor management of domestic dogs, roaming stray dogs, lack of regular vaccination of dogs, poor management of patients after dog bites, in terms of rapid and thorough washing of the wound, completion of post-exposure vaccination schedules plus inoculation with *rabies* immunoglobulin for severely exposed bite-victims results in the disease claiming upto 24,000 human deaths annually in Africa alone (Tschopp *et al.*, 2016).

LSDV is one of the species of Capripoxviruses affecting cattle. The virus is resistant to different chemical and physical agents. The virus can persist for about 33 days in necrotic skins and remain viable for at least 18 days in lesions in air-dried hides at ambient temperature. LSDV is very resistant in the environment and can remain viable for long periods on or off animal hosts, persisting up to six months in a suitable environment, such as shaded animal pens. Capripoxviruses have lipid-containing envelopes and susceptible to a range of disinfectants containing detergents. The virus contaminates the environment through shedding via nasal, lachrymal and pharyngeal secretions, semen, milk and blood. It may remain in saliva for up to 11 days and in semen for 22 days (Hailu *et al.*, 2015)

Capripoxviruses causing SGP enter via the respiratory tract and transmission commonly is by aerosol infection associated with close contact with infected animals. Spread can also occur from contact with contaminated materials and through skin abrasions produced iatrogenically or by insects. Viruses are shed in

secretions and excretions of infected animals. Movement of infected animals acts as the main cause of spreading SGP viruses (Mirzaie *et al.*, 2015). For Newcastle Disease virus, transmission of virus infection from one bird to another is normally through contamination of the environment via shedding of virus in faeces. This is influenced by environmental factors, such as temperature, humidity and stocking density (Alexander, 2000).

Diseases were ranked according to feasibility of their control methods as well. *Brucellosis* was ranked highest, followed by HPAI and RVF. Normally when human *brucellosis* is significant and the incidence of *brucellosis* in animals is quite high leading to significant economic losses in terms of reducing meat and milk outputs, *brucellosis* is considered a high priority disease, hence easily attracts attention in terms of control. Experience has shown that interventions to control and eradicate *brucellosis* need to be based on engagement with livestock keepers, education and complementary measures and, where feasible and necessary, vaccination. Tailoring interventions on the impact of the disease in terms of magnitude of burden in potential animal and human hosts is thus essential. Control relies on three vaccines marketed worldwide: B. abortus S19 and B. abortus RB51 against *brucellosis* in cattle and Melitensis Rev 1 against *brucellosis* in small ruminants (Ducrotoy *et al.*, 2015).

For HPAI, methodologies and technologies for its control are available and accessible. Active, targeted surveillance following the diagnosis of HPAI infection coupled with culling of infected birds at-source and strict biosecurity measures have proved to be effective in the control and eradication of the disease. Highly efficacious, safe and affordable vaccines are commercially available. Success stories in many countries on the control and eradication of HPAI infections in Europe (Italy and the Netherlands) and North America (Mexico, USA and Canada) has proved that control and eradication of HPAI is feasible. For example, HPAI H5N1 outbreaks have been stamped out in Hong Kong, Japan, Republic of Korea, DPR Korea and Malaysia through

enhanced surveillance, strict biosecurity measures and culling of infected poultry (FAO/OIE/WHO, 2005).

Control of RVF using a combination of (i) control of livestock movements with respect to trade and export; (ii) vector control with an emphasis on larvicides in vector breeding sites and (iii) vaccination of livestock has proved to be effective. Besides trade control, a safe vaccine is now available for livestock-an efficient way to protect both animals and humans interrupting the virus transmission in endemic areas. Creating awareness among people regarding appropriate slaughtering and consumption practices decreases the risk of infection to humans since direct exposure to infected animals is the major route of infection to humans (Balenghien *et al.*, 2013).

Furthermore, diseases were ranked according to their ecological impact as regards their mode of transmission. HPAI, followed by RVF and PPR were ranked highest. HPAI is a highly infectious and dynamically evolving disease that spreads rapidly and widely. The presence of free-grazing ducks is known to influence occurrence of HPAI outbreaks in domestic poultry. Domestic ducks in endemic areas normally harbour HPAI virus hence preventing the intermingling of ducks and domestic poultry would serve to significantly reduce HPAI transmission. Wild birds as well play a role in the transmission of H5N1 viruses to domestic poultry. Migratory bird species as well act as carriers, and can transport HPAI over longer distances (FAO/OIE/WHO, 2005).

Rift Valley fever virus (RVFV) is transmitted among ruminants by mosquito bites mainly belonging to the *Aedes* and *Culex* genera and by direct contact with body fluids of viremic animals. Humans are mainly infected by close contact with blood, excreta of infected animals, consumption of raw milk and in some rare cases, through mosquito bites. RVFV circulates between animals within an enzootic cycle during most years, but may become epizootic during wet years in regions. Usually the virus is maintained during dry seasons in desiccation-resistant eggs of several *Aedes* species which have acquired the

virus by vertical transmission. For example, in East Africa, flooding of natural excavations leads to the hatching of large numbers of *Aedes* (*Aedimorphus* and *Neomelanicion* subgenera) eggs initiating viral circulation. Movements of viremic animals along trade routes have been suspected to be responsible for the virus spreading (Balenghien *et al.*, 2013).

PPR is highly contagious when it first occurs in a naïve population. Periodic outbreaks may be seen in endemic regions, particularly when animals are mixed or new animals are introduced into a herd. Often epizootics are associated with changes in weather, such as the beginning of the rainy season or a cold, dry period. Circulation sub-clinically in small ruminant populations coupled with emergence when immunity wanes or naïve animals are introduced are thought to be the main means of maintenance of the virus between outbreaks. A wide host range involving sheep, goat, camels and water buffalo coupled with high morbidity and case fatality rates in small ruminants, especially in naïve herds that reach 80-90% or greater causes a high ecological impact (The Centre for Food Security and Public Health, 2015).

Overall RVF, followed by CBPP, NCD, HPAI, LSD, PPR, *Rabies*, *Brucellosis*, *Bovine tuberculosis*, FMD and SGP were ranked highest within the IGAD. Similarly, earlier prioritization exercises based on perceived economic impact on trade, transmissibility, zoonotic potential, and disease prevalence identified FMD, CBPP, PPR, RVF, *Brucellosis*, SGP, LSD, CCPP and Camel Pox as the nine priority notifiable diseases in IGAD (RISP II, 2013).

Country-specific prioritization of the diseases was considered for Djibouti, Ethiopia, Eritrea, Somalia, South Sudan and Sudan. For Djibouti, the status for several important diseases such as *Rift Valley fever*, Sheep and Goat Pox, Camel Pox and Newcastle Disease in Djibouti was unknown (PAAHYB, 2012; PAARYB, 2013; PAARYB, 2104). However, six diseases were identified as top priorities given impending risk of outbreaks as a result of transit livestock passing through Djibouti. Amongst which PPR and CCPP were

considered important due to the significance of small ruminants in Djibouti. CBPP was also considered an important disease for the country that could justify vaccination campaigns. In addition, *Brucellosis*, a zoonotic disease was considered a disease of great economic importance and as well a human health issue. FMD was considered as an important trade sensitive disease requiring early detection and rapid response. *Bovine tuberculosis* was also considered as an important zoonotic disease.

For Ethiopia, PPR, LSD and FMD, were identified as top priorities in line with existing records (PAARYB, 2014). PPR was the first priority disease due to the importance of small ruminants in the country, while LSD is an important disease of significant economic impact on livestock production and creates hindrance to trade. FMD is also a priority disease for the country both for the economic impact on production and the impact on trade of live animals and animal products. *Brucellosis* and *Bovine tuberculosis* were considered as important zoonotic diseases too. For priority absent diseases, RVF was the top priority taking into account the presence of the virus in neighboring countries.

For Eritrea, PPR, FMD, SGP, ND, *Brucellosis* and *Bovine tuberculosis* were the top priority diseases. In accordance with existing records, outbreaks of PPR, FMD, SGP, ND and *Brucellosis* were reported in Eritrea in 2014 (PAARYB, 2014). PPR was selected due to the importance of small ruminants in the country, while FMD due to its impact on trade and animal production. SGP was selected due to its impact on trade of small ruminants. Newcastle disease was considered a major hindrance to poultry production, a major source of livelihood for the poorest population. *Brucellosis* and *Bovine tuberculosis* were considered two important zoonotic diseases.

For Somalia, CCP, SGP, *Brucellosis* and FMD were considered top priorities. Existing records too confirmed outbreaks of these diseases as per reports from Somalia in 2014 (PAARYB, 2014). CCP and SGP were considered important, given the importance of small ruminants in Somalia. *Brucellosis* was

considered an important zoonotic disease, while FMD was considered a priority disease given its economic importance.

For South Sudan, FMD, PPR, RVF, *Brucellosis* and CBPP were identified as top priorities. Existing reports indicate that outbreaks of FMD, PPR and CBPP were recorded in South Sudan in 2014 (PAARYB, 2014). FMD was considered important given the free movement of livestock between South Sudan and neighboring countries. PPR was considered important given the significance of the small ruminant production. RVF had been present and reported in humans several years ago. However, new cases have not been reported ever since that time. *Brucellosis* was considered an important zoonotic disease given a large proportion of the population that drinks raw milk in the country. CBPP was considered a priority disease due to the importance of cattle and the high risk.

For Sudan, FMD, PPR, Sheep and Goat Pox, CBPP, *Brucellosis*, *Rabies* and RVF were identified as top priorities. Existing records confirmed outbreaks of FMD, PPR, SGP, CBPP, *Brucellosis* and *Rabies* were reported in Sudan in 2014 (PAARYB, 2014). FMD is a multispecies disease creating important losses in the country, and hinders trade. PPR and SGP are important due to high population of small ruminants. Meanwhile, CBPP is an important disease of cattle. *Brucellosis* and *Rabies* were considered important zoonotic diseases in Sudan, while RVF was considered a priority given its presence in the neighbouring Egypt.

## Conclusion

It is anticipated that the prioritization of TADs and zoonoses for the IGAD region will facilitate systematic efforts to roll back priority diseases through focused national efforts, harmonized regional strategies and streamlining of stakeholder investment in the region's main priorities. Furthermore, it is necessary to generate appropriate data on disease impact in order to mobilize required support among decision makers and other stakeholders. The effective control of infectious diseases depends

on surveillance, preventive measures and when necessary outbreak investigation and the institution of control measures. Developing and sustaining such measures at national level requires a substantial long-term commitment in terms of human, financial and material resources by national governments.

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## SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF BRUCELLOSIS IN DAIRY CATTLE IN SELECTED TOWNS OF WEST SHEWA, ETHIOPIA

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### Abstract

*Bovine brucellosis* is a contagious disease of cattle causing reproductive failure, loss of milk production and zoonosis worldwide. A cross-sectional epidemiological study was conducted on 816 dairy cattle (449 were cows) from 60 dairy farms to determine the seroprevalence and associated risk factors of *bovine brucellosis* in dairy cattle in selected towns of West Shewa, Ethiopia. Sera were collected, screened by Rose Bengale Plate Test and positive sera were further tested by Complement Fixation Test for confirmation of *Brucella* seropositivity. Data regarding risk factors were obtained from records and questionnaire. The association of *brucellosis* with risk factors was analyzed by Chi-square/Fischer's exact test. The result showed 0.49% (4/816), 0.9% (4/449) and 3.3% (2/60) seroprevalence in cattle, cows alone and at herd level, respectively. Among the risk factors herd size ( $X^2=4.24$ ), history of abortion (OR=8.94) and retained fetal membrane (OR=8.39) showed significant association ( $p<0.05$ ). The results of questionnaire survey showed 90% of the respondents do not know about *brucellosis* and use no maternity pen for their cows and 86.7% of them use no safety measure during assisting delivery and disposing afterbirth materials. The prevalence of *bovine brucellosis* in the present study was low; however, the risk attributed by a single carrier cow to the public health is high in the presence of lack of awareness and poor hygienic practice. Therefore, at this prevalence level to test and cull seropositive cows at farm level and creating public awareness is suggested.

**Key words:** *Brucellosis*, Dairy cattle, Seroprevalence, Risk factor, West Shewa

## SÉROPRÉVALENCE DE LA BRUCELLOSE ET FACTEURS DE RISQUE ASSOCIÉS CHEZ LES BOVINS LAITIERS, DANS CERTAINES VILLES DE L'OUEST DE SHEWA EN ÉTHIOPIE

### Résumé

La brucellose bovine est une maladie contagieuse qui est à l'origine de l'infertilité, de la perte de production de lait et de zoonoses, partout dans le monde. Une étude épidémiologique transversale a été réalisée sur 816 bovins laitiers (449 vaches) issus de 60 fermes laitières, dans le but de déterminer la séroprévalence de la brucellose bovine et les facteurs de risque associés chez les bovins laitiers de certaines villes de l'Ouest de Shewa en Éthiopie. Des sérums ont été prélevés, étudiés au moyen du test au rose bengale, et les sérums qui se sont révélés positifs ont fait l'objet d'un examen plus approfondi utilisant le test de fixation de complément pour confirmer la séropositivité à la *Brucella*. Les données relatives aux facteurs de risque ont été obtenues à partir des dossiers et d'un questionnaire administré. L'association de la brucellose aux facteurs de risque a été analysée en utilisant le test de Chi-carré / méthode exacte de Fischer. Le résultat a montré une séroprévalence de 0,49% (4/816), 0,9% (4/449) et 3,3% (2/60) respectivement chez les bovins, les vaches seules et au niveau du troupeau. Parmi les facteurs de risque, la taille du troupeau ( $X^2 = 4,24$ ), les antécédents d'avortement (OR = 8,94) et la rétention des membranes fœtales (OR = 8,39) ont montré une association significative ( $p < 0,05$ ). Les résultats de l'enquête par questionnaire ont révélé que 90% des répondants ne savaient rien de la brucellose et n'avaient pas d'enclos de vêlage pour leurs vaches et que 86,7% d'entre eux n'utilisaient aucune mesure de sécurité pendant la mise-bas et l'élimination du placenta après la mise-bas. Le taux de prévalence de la brucellose bovine dans la présente étude était faible; cependant, le risque que constitue une seule vache

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porteuse pour la santé publique est élevé compte tenu du manque de sensibilisation et de l'application de mauvaises pratiques d'hygiène. Par conséquent, avec ce taux de prévalence, il est proposé de procéder au diagnostic et à l'élimination de vaches séropositives au niveau de la ferme et à la sensibilisation du public.

**Mots-clés :** brucellose, bovins laitiers, séroprévalence, facteur de risque, Ouest de Shewa

## Introduction

*Bovine brucellosis* is a major zoonotic disease widely distributed in both animals and humans especially in the developing world. It is a disease of cattle usually caused by *Brucella abortus*, on occasionally by *Brucella melitensis* and rarely by *Brucella suis* (Radostitis et al., 2007). The disease impacts both animal and human health, as well as economic consequences, especially in countries where livestock production play the greatest economic role (Roth et al., 2003).

*Bovine brucellosis* is a contagious infectious disease of cattle characterized primarily by abortion in late stage of pregnancy, frequently followed by retention of fetal membrane and endometritis, which may cause infertility in subsequent pregnancies (Al-Majali et al., 2009). The common signs in male are orchitis and epididymitis with frequent infertility (Radostitis et al., 2007). Ingestion of feed and water contaminated by brucella organisms from aborted fetus and fetal materials is the common means of transmission to susceptible animals (Al-Majali et al., 2009). However, it has been reported that infection through injured/intact skin, the mucosa of the respiratory system, and conjunctiva is not uncommon (Kebede et al., 2008). Semen from infected bulls, if inseminated by artificial means, it can definitely transmit the infection to the cows (De Massis et al., 2005).

The disease in man (undulant fever) is transmitted through consumption of raw milk and its products or through contact with afterbirth products from infected animals (Khorasgani et al., 2008). The disease is acute febrile in man which may progress to a more chronic form and can also produce serious musculo-skeletal, cardiovascular and nervous complications (OIE, 2009).

Currently the prevalence of *bovine brucellosis* is highest in dairy cattle in Latine

America and developing countries like in Africa, though the rate of infection varies greatly among the countries. However, most European countries are free of *bovine brucellosis* (Seifert, 1996). In Ethiopia, the dairy production has been growing to meet an ever increasing demand for milk and milk products. Cross breeding indigenous cattle with high yielding exotic cattle is used to bridge the gap between supply and demand for dairy products (Deselegn and Gangwar, 2011). Owners of dairy cattle and institutions promoting the dairy industry require current reliable information on important diseases such as brucellosis. The unlikelihood of treatment and economic loss through abortion, reduction of milk yield and loss of fertility could impede the developing dairy sector and risk to public health shows that the disease is highly important. Studies on *bovine brucellosis* in various parts of the country have showed the prevalence of the disease at various rates. However, no similar work concerning *bovine brucellosis* has been conducted in districts of west Shewa zone so far. Moreover, according to the data from district veterinary clinics and artificial insemination technicians, there have been several repeated complaints for poor reproductive performance by dairy cattle owners. Therefore, the objective of this study was to determine the seroprevalence and associated risk factors of *bovine brucellosis* in dairy cattle in selected towns of west Shewa Zone, Ethiopia.

## Materials and methods

### *Description of the study area*

The study was conducted in five selected towns namely Ambo, Holeta, Addis Alem, Ginchi and Guder of West Shewa Zone, Ethiopia from October 2013 to January 2014. Ambo is the administrative center for the Zone, which is located at 114 km west of Addis Ababa.

The other four towns are the administrative towns for their respective district (woreda). According to the zonal basic data, the zone is dominated by high land environment followed by mid altitude and small share of low land. All the study towns are dominated by high land and mid altitude, which is good climatic condition for the adaptation of exotic breeds of dairy cattle. As a result the establishment of dairy farms and small holders targeting milk production is increasing in the area.

#### *Target population and study Animals*

The target populations were crossbred dairy cattle in urban and peri-urban areas kept for commercial purpose in the selected towns. List of dairy farm owner was obtained from the respective districts livestock agency from which volunteer dairy farm owners holding at least 3 cross breed cattle in their herd were included in the study. Cattle kept under small (holding less than 30) and medium (holding 30 and above) dairy farm, managed in either intensive or extensive system and selling raw milk for the community were included in this study. The study animals were cross breed dairy cattle with the age of 6 months or above, which belonging to volunteer dairy farmer/ farm owners.

#### *Sampling method and study design*

Sixty volunteer dairy owners were selected and a total of 816 dairy cattle owned by these owners were sampled to screen *bovine brucellosis* using cross-sectional study. Screening of sera was performed using Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) was used for confirmation of RBPT positives sera. In addition survey was conducted using a semi-structured questionnaire, and 60 dairy owners were interviewed to assess the different risk factors such as herd composition, herd size, breeding system, animal management, knowledge about brucellosis, hygienic disposal of aborted material and presence of maternity pen. Before the actual interview and blood sample collection verbal consent was obtained from each farmer. Data regarding history of reproductive failure (poor breeding

performance rate, abortion, retained fetal membrane) in the herd were also compiled.

#### *Ethics statement*

The seroprevalence study involves interview with dairy cattle owners and blood sampling from dairy cattle. The study protocol was assessed and approved by the Ambo University Animal Research Ethics Review Committee (ARERC) on December 10, 2012, with its reference number RCCSD/AREC/002/2012. The dairy farmers were informed about the purpose and the methods of the study and informed consent was obtained from all individual participants included in the study.

#### *Blood sample collection and serological analysis*

Approximately 10ml of blood sample was collected from the jugular vein of each cattle using sterile needle and plain vacutainer tube and labeled. The collected sera were allowed to clot overnight in a slant position at room temperature. After 24 hrs, sera were separated from the clot into cryovials by decanting; unretracted blood was centrifuged to separate serum. Finally the sera samples were stored at -20°C until tested. All sera samples were screened by RBPT, using an antigen suspension consisting of *B. abortus* according to the procedures described by the OIE (2012) at Ambo University veterinary laboratory. Thirty micro liters of serum was mixed with an equal volume of antigen suspension on a plate and agitated. After 4 minutes of shaking, visible agglutination was considered as a positive result. The test was repeated when test results were unclear. Sera positive for the RBPT were further tested using CFT complying with the standard protocol OIE (2012) at National Veterinary Institute, Debre Zeit, Ethiopia. A standard *B. abortus* antigen for CFT was employed to detect the presence of antibodies against *Brucella* in the sera. The control sera and complement are both obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany.

### Data management and analysis

Data obtained both from serological tests and questionnaire survey were entered into Microsoft excel spreadsheet and analyzed using the SPSS 20.0 statistical package (SPSS Inc., Chicago, Illinois, USA). Animals tested positives to both RBPT and CFT were defined as seropositive and herds having at least one seropositive cattle were considered positive. Animal level seroprevalence was computed by dividing the number of positive animals by total number of animals tested and for herd level seroprevalence the number of positive herd was divided to the total number of herds tested. Data from questionnaire was summarized by simple descriptive way. The associations of serological status of *bovine brucellosis* with risk factors such as age, sex, herd size, parity, breeding system, management system and study towns as well as history of reproductive disorders was assessed using Chi-square test/ Fischer's exact test. For statistical inference 0.05 was considered as level of significance.

### Result

Sixty small and medium level dairy farms from five towns namely Ambo (N=20), Holeta (N=15), Ejere (N=9), Guder (N=8) and Ginchi (N=8) were investigated. Out of the total 816 sera samples tested, the overall individual animal level seroprevalence was 0.49% (4/816). All the seropositive cattle were cows 0.9 % (4/449) and the herd level seroprevalence was 3.3% (2/60). Individual animal level seroprevalence was 1.08% (3/277) at Ambo, 1% (1/285) at Ejere and 0% in the rest of towns. Herd level seroprevalence of 11.1% (1/9), 5% (1/20), and 0% was recorded at Ejere, Ambo and the rest the towns, respectively. However, the variation among the towns was not statistically significant (Table 1).

Among the risk factors only herd size showed marginally significant association ( $p < 0.05$ ), with cattle in herds holding  $> 30$  had higher prevalence 1.01% (4/390) to those holding less than 30 cattle 0% (0/426). The other factors namely age, sex and management system did not significantly influence the

occurrence of brucellosis infection. However, the seroprevalence was observed to be numerically higher in intensively managed animals 0.75% (3/401); in cattle older than 6 years of age 1.37% (3/219) and only in female animals 0.53 % (4/756) as compared to their counterpart (Table 2).

Seroprevalence of *bovine brucellosis* in cattle alone was significantly associated with history of abortion and retained fetal membrane with higher risk of acquiring infection in cows with history of abortion (OR=8.94,  $p < 0.05$ ) and retained fetal membrane (OR= 8.39,  $p < 0.05$ ). The remaining factors namely parity, breeding system and history of poor breeding performance did not show significant association ( $p > 0.05$ ) with brucellosis seroprevalence. However, a numerically higher seroprevalence as compared to their counterpart was recorded; 1.47% (3/204) in dairy cows having more than 2 calves, 1.31% (4/306) in cows served either by bull or AI and 1.30% (2/154) in cows with poor breeding performance rate (Table 3).

The result of questionnaire survey indicated 90% (54/60) of the respondents did not have any information regarding brucellosis and 86.67% (52/60) did not use protective precaution while assisting animals with difficult parturitions and discarding aborted fetus and fetal membrane. Moreover, only 10% (6/60) of the respondents had maternity pen for their dairy herd (Table 4).

### Discussion

The low overall individual animal level seroprevalence (0.49%) of brucellosis in the present study was comparable with 0.4% report of Asmare et al. (2007) from urban dairy farms of Northern Ethiopia and Sebeta and 0.5% Tolosa et al. (2010) and Degefu et al. (2011) from cattle managed under extensive system. Relatively higher prevalence were reported in dairy cattle by other researchers; for instance seroprevalence of 1.5%, 1.7%, 2.5%, 3.9% and 10% were reported by Tesfaye et al. (2011); Tschopp et al. (2013); Asmare et al. (2007); Abebe et al. (2009); Eshetu et al. (2005),

**Table 1:** Individual and herd level prevalence of bovine brucellosis in the study towns

Towns	No. of animals tested	No. of CFT positive cattle (%)	No. herd tested	No. of CFT positive herd (%)
Ambo	277	3 (1.08)	20	1 (5.00)
Guder	116	0	8	0
Holeta	285	0	15	0
Ejere	100	1 (1.00)	9	1 (11.11)
Ginchi	38	0	8	0
<b>Total</b>	<b>816</b>	<b>4 (0.49)</b>	<b>60</b>	<b>2 (3.33)</b>

$\chi^2=4.69, p>0.05$  (individual level)  $\chi^2=3.01, p>0.05$  (Herd level)

**Table 2:** Association of seroprevalence of bovine brucellosis with risk factors

Factor	Category	Total No. examined	CFT positive	Chi- square	P-Value
Management system	Intensive	401	3 (0.75)	4.16	0.12
	Semi-intensive	358	1 (0.28)		
	Extensive	57	0		
Age	<2years	272	0	5.04	0.08
	2-6 years	324	1 (0.31%)		
	>6 years	219	3 (1.37%)		
Sex	Male	60	0	0.32	0.736
	Female	756	4 (0.53%)		
Herd size	<30 animals	426	0	4.24	0.048
	> 30 animals	390*	4 (1.03%)		

<: less than, >: greater than, \*:  $P<0.05$

**Table 3:** Association of bovine brucellosis seroprevalence with the risk factors in dairy cows

Risk factor	Category	Total No. examined	Brucellosis positive	Odds Ratio	95% CI	P-Value
Parity	<2	245	1 (0.41%)	3.64	0.37-35.28	0.26
	>2	204	3 (1.47%)			
Breeding method	Only AI	143	0 (0.70%)	0.92	0.894-1.018	0.761
	Mixed	306	4 (1.00%)			
History of abortion	Absent	432	3 (0.69%)	-	-	-
	Present	17	1 (5.88%)	8.94	0.88-90.72	0.026
History of retained fetal membrane	Absent	431	3 (0.70%)	-	-	-
	Present	18	1 (5.56%)	8.39	0.83- 84.93	0.032
History of poor breeding performance	Absent	295	2 (0.68%)	-	-	-
	Present	154	2 (1.30%)	0.52	0.07- 3.72	0.506

<: less than, >: greater than, CI: confidence interval, AI: Artificial insemination

**Table 4:** Result of questionnaire survey concerning knowledge and practice of brucellosis

Knowledge and practice	Category	Percent of farms/owners
Knowledge about brucellosis	Yes	6/60 = 10%
	No	54/60 = 90%
Precaution during assisting delivery and handling aborted fetus and fetal membrane	Yes	8/60 = 13.3%
	No	52/60 = 86.7%
Presence of maternity pens	Yes	6/60 = 10%
	No	54/60 = 90%

respectively. Even higher prevalence of 39% was recorded by Meyer (1980) while Asmare *et al.*, (2011) from Nazareth, Gondar and Mekele; Belihu (2002) from Salale and Addis Ababa and Alem and Solomon (2002) from central Ethiopia were unable to find a single positive reactor in intensive dairy farms. The herd level seroprevalence of 3.3% in the present study was also lower compared to 15%, 42.31% and 45.9% the report by Ibrahim *et al.* (2010); Berhe *et al.* (2007) and Kebede *et al.* (2008), respectively. The lower individual animal and herd level seroprevalence in the current study might be due to culling of seropositive dairy cattle owned by private and government farms based on recommendation given by regional veterinary laboratory following screening *bovine brucellosis* in 2010 (personal communication). Moreover this could also be indicative of less level of intensification of the dairy farming in the present study areas as compared to central high lands of Ethiopia. However, the variation in seroprevalence reported by different authors might be due to variation in breed of study animals, level of management intensification, herd size, breeding system, and serological tests employed (Hailesilasie *et al.* 2010; Tesfaye *et al.* 2011).

The numerically higher seroprevalence in intensive dairy farm than the semi-intensive and extensive in the present study is in agreement with the reports of Bekele *et al.* (2000) and Hailesilasie *et al.*, (2010). In line with this it has been well documented that the level of brucellosis infection tends to be higher in intensive farms and the risk of infection increase with intensification when

aborting cows are present in the herd and hygienic practices is low (FAO/WHO, 1986). This could be due to higher chance of contact between healthy and infected animals in these systems and inattention of some farmers to segregate infected animals and proper disposal of infectious materials.

It is well known that sexually mature animals are more susceptible to *Brucella abortus* infection (Radostitis *et al.*, 2007). Accordingly a fairly higher seroprevalence in cattle of older than 6 years and seronegativity in animals below 3 years old was observed in the present study. The same trend has been reported by Hailesilasie *et al.* (2007), Abebe *et al.* (2008) and Degefu *et al.* (2011).

Although there is disproportional sampling of male ( $n=60$ ) over female ( $n=256$ ) in the present study, the absence of male reactors is in agreement with the findings of Asmare *et al.*, (2007), which might be due to the limited serological response of male animals to *Brucella* infection because of low antibody titer of testes of infected males (Crawford, 1990).

In the current study the proportion of seropositive animals was significantly higher in herds holding greater than 30 animals (1.03%) than those holding less than 30 animals (0%), which is in agreement with the report of similar authors (Hailesilasie *et al.*, 2007; Asmare *et al.*, 2007). This could be due to the cumulative effect of intensive management system and stocking density, which facilitate rate of contact in dairy herds.

It is well-known fact that in susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature

of bovine brucellosis, and retention of fetal membrane and metritis are common sequels to abortion (Walker, 1990; Schelling, 2003). Different researchers have reported significant association between history of abortion and retained fetal membrane with brucellosis (Al-Majali, *et al.*, 2009; Deselegn and Gangwar, 2011; Berhe, *et al.*, 2007). In line with this the present study revealed significant association ( $P < 0.05$ ) of *brucellosis* seropositivity with history of abortion and retained fetal membrane, though, the number of cows with history of abortion ( $n=17$ ) and retained fetal membrane ( $n=18$ ) were small. This could be due to the reason that some farmers could hide of cows with history of abortion and retained fetal membrane for fear of risking acceptance of their dairy product as their lively hood is based on the income they generate from milk and milk products. Moreover, this study considered two years information, since cows usually abort once perhaps at the first pregnancy (OIE, 2009), cows might have aborted in their earlier calving and their number might be small. The cause of abortion and retained fetal membrane in seronegative cow with history of abortion and retained fetal membrane might be other factors such as malnutrition, deficiency diseases and other infections. According to Asmare *et al.* (2013) abortion, retained fetal membrane and poor breeding performance were more associated with *Neospora* than *Brucella* in breeding dairy herds.

*Brucellosis* in humans is an occupational hazard to animal attendants, slaughter house workers and veterinarians (Radostitis *et al.*, 2007; OIE, 2009). In line with this, the lack of information regarding brucellosis in 90%, absence of maternity pen in 90% and poor hygienic practice in 86.67% of the respondents in the current questionnaire surveys showed the increased zoonotic risk of brucellosis for the dairy personnel in the study area.

In conclusion the present study detected brucellosis in two out of the five studied towns and large herd, presence of history of abortion and retained fetal membrane were the detected risk factors. Although the seroprevalence is low, the lack of

awareness about the disease and poor hygienic practices in the preset study alarms the need for due attention to reduce public health risk. Hence, eliminating carrier dairy cows is suggested at this prevalence level together with raising public awareness regarding brucellosis. Moreover, beyond the epidemiological survey, detection and typing the prevailing strains of *Brucella* is recommended to aid its control at national level.

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## SINGLE PHASE FEEDING OF FISHMEAL AND ITS INFLUENCE ON GROWTH AND CARCASS OF BROILER CHICKENS

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### Abstract

A 63 days study was conducted at the poultry experimental station of Abubakar Tafawa Balewa University Bauchi, Nigeria. In the study, one hundred and ninety-two (192) day-old mixed-sexed broiler chicks of strain Arbor Acres were randomly allotted to four dietary treatment groups with different fish meal inclusion levels (0, 1.5, 2.5, and 3.5 %) for treatment A, B, C and D respectively. Each treatment had four replicates with twelve birds per replicate in a completely randomized design. The initial body weight, final body weight, total weight, daily feed intake, total feed intake and feed conversion ratio had no significant ( $P > 0.05$ ) effects when other diets (treatments B, C and D) were compared to the control (treatment A). Likewise, all carcass parameters were not significantly affected ( $P > 0.05$ ) by the dietary treatments except the kidney. Kidney of birds fed diet B (1.5 %) fishmeal inclusion level was significantly ( $P < 0.05$ ) higher than that of the birds on other treatments. LSD was used to separate the means. No mortality was recorded throughout the study period. It can be concluded that, inclusion of fishmeal in diets of broiler chickens using a single phase feeding should not exceed 1.5 % for optimum performance and efficient use of resources.

**Key words:** Broiler, Chickens, Fishmeal, Carcass, Growth and Single-phase.

## ALIMENTATION EN PHASE UNIQUE À LA FARINE DE POISSON ET SON INFLUENCE SUR LA CROISSANCE ET LA CARCASSE DES POULETS DE CHAIR

### Résumé

Une étude de 63 jours a été réalisée à la station expérimentale de volailles de l'Université Abubakar Tafawa Balewa à Bauchi au Nigeria. Dans l'étude, cent quatre-vingt-douze (192) poussins de chair de la souche Arbor Acres des deux sexes, âgés d'un jour, ont été affectés de manière aléatoire à quatre groupes de traitement diététique comportant différents niveaux d'inclusion de farine de poisson, à savoir 0 ; 1,5 ; 2,5 et 3,5%, respectivement pour les traitements A, B, C et D. Chaque traitement comportait quatre répétitions avec douze oiseaux par répétition, dans un schéma complètement aléatoire. Le poids corporel initial, le poids corporel final, le poids total, la consommation alimentaire journalière, la consommation alimentaire totale et l'indice de consommation n'ont pas montré d'effets significatifs ( $P > 0,05$ ) au moment de la comparaison des autres régimes (traitements B, C et D) avec le traitement témoin. De même, aucun paramètre de la carcasse n'a été significativement affecté ( $P > 0,05$ ) par les traitements diététiques à l'exception du rein. Les paramètres des reins des oiseaux soumis au régime B (1,5%) étaient significativement ( $P < 0,05$ ) plus élevés que ceux des oiseaux soumis aux autres traitements. La LSD a été utilisée pour comparer les moyennes. Aucune mortalité n'a été enregistrée tout au long de la période d'étude. On peut conclure que l'inclusion de farine de poisson aux régimes alimentaires des poulets de chair par alimentation en phase unique ne devrait pas dépasser 1,5% pour une performance optimale et une utilisation efficace des ressources.

**Mots-clés :** poulet de chair, poulets, farine de poisson, carcasse, croissance et phase unique

## Introduction

Continued effort towards reducing cost of producing poultry is being made by researchers (Eyng *et al.*, 2013). Fishmeal is commonly used to increase palatability, level of protein and appreciable quantities of fat and minerals (Karimi, 2006). Fishmeal has high biological value in diets of animals, with values of crude protein reported at 65 %, crude fibre 1 %, ash 18 %, ether extract 4.5 %, calcium 6.8%, phosphorus 3 % (Aduku, 1993). It is rich in essential amino acids, particularly lysine methionine and cysteine thereby supplementing deficiencies in vegetable protein sources such as soybean meal (Miles and Jacob, 1997). Diets with varying levels of protein and energy levels have been recommended for single phase feeding in broiler chicken production by various researchers. Oyedeji, *et al.*; (2005) recommended 18 % crude protein and 3200 kcal/kg energy for both broiler starter and finisher phase (single diet) while Aremu *et al.*; (2011) recommended a diet of 24 % crude protein and 2600 kcal/kg energy for single diet. The objective of the study was to investigate the best levels of fishmeal in a single phase feeding regimen of broilers because there is no agreeable standard that is required.

## Materials and methods

The study was conducted at the poultry experimental station of Abubakar Tafawa Balewa University Bauchi State, Nigeria. Bauchi lies within the guinea savannah zone of Nigeria, which is characterized by two distinct (dry and rainy) seasons. The dry season starts in October and ends in April while the rainy season spans from May to September. The maximum rainfall and temperature recorded were 1300 mm per annum and 37.60 C respectively (NIMET, 2015).

One hundred and ninety-two day-old unsexed broiler chicks of strain Arbor Acres were purchased from Zartech Farms, Jos, for the study. The study lasted for nine weeks, comprising seven days of brooding and eight weeks (56 days) of data collection.

At one week of age, the chicks were randomly allotted to four treatment groups (0, 1.5, 2.5, and 3.5 %) having different levels of fishmeal inclusion. Each treatment (A, B, C and D) had four replicates and each replicate had twelve birds allotted in a completely randomized design. The birds were brooded on deep litter in sixteen different pens (each measuring 2.7 m<sup>2</sup> by 1.2 m<sup>2</sup>) which provided a floor space of 0.27 m<sup>2</sup> per bird. Four iso-nitrogenous and iso-caloric diets (21.05 %CP; 2900 Kcal/kg ME) were formulated to meet nutrient requirement standards of broilers (NRC, 1994) for all nutrients (Table 1). Experimental diets were formulated to contain different fishmeal levels (0, 1.5, 2.5 and 3.5% during starter and finisher period). The diets and water were offered *ad-libitum* throughout the study period. Drinkers and feeders were cleaned daily before fresh feed was offered. Also, strict hygiene was ensured daily in order to maximise comfort for the chicks. Table 1 shows the composition of ingredients used within each treatment:

Birds in each treatment were weighed at the beginning of the experiment and on weekly basis thereafter to determine the weight gain of birds. Feed intake was recorded daily and was determined by obtaining the difference between quantities of feed offered the previous day and the left over the following morning. Feed conversion ratio was calculated from the data on feed intake and weight gain as the number of grams of feed taken per gram of weight gained over the same period. At the end of experimental period, 8 birds were randomly selected from each group for carcass evaluation. Each bird was starved overnight, weighed and slaughtered; they were then scalded, plucked and eviscerated. The carcass and internal organs (liver, heart, kidney, gizzard, lung, liver, abdominal fat and spleen) were removed, weighed and expressed as a percentage of live weight according to 'Modified Kosher' method as described by Abe *et al.*, (1996). Data were subjected to one-way analysis of variance (ANOVA) to determine significance of the treatment effects using Minitab 15 software. The means were compared using individual 95% confidence interval test generated by the Minitab software.

**Table 1:** Composition (%) of the experimental diets

Parameters	Inclusion levels of fishmeal (%)			
	A (0.00)	B (1.5)	C (2.5)	D (3.5)
Maize (Yellow)	53.57	54.43	55.00	55.58
Groundnut Cake	32.28	29.92	28.35	26.77
Fishmeal	0.00	1.50	2.50	3.50
Maize Offal	9.00	9.00	9.00	9.00
Bone Meal	3.50	3.50	3.50	3.50
Lime stone	0.50	0.50	0.50	0.50
Common Salt	0.30	0.30	0.30	0.30
Vitamins/Minerals Premix	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
<b>Calculated analysis (%)</b>				
Crude Protein	21.05	21.05	21.05	21.05
ME (Kcal/kg)	2,903.00	2,914.00	2,921.00	2,929.00
Crude Fibre	3.77	3.69	3.63	3.77
Phosphorus	0.64	0.68	0.71	0.73
Calcium	1.52	1.61	1.67	1.73
Lysine	1.02	1.05	1.07	1.09
Methionine	0.52	0.54	0.55	0.56

### Results

Results on growth performance of broiler chickens fed fishmeal at varying levels of dietary inclusion for 56 days is presented in Table 2, while results for carcass characteristics and internal organs are presented in Table 3.

### Discussion

Table 2 shows the growth performance of broiler chickens fed fishmeal at varying levels of dietary inclusion for 56 days. There were no significant differences ( $P>0.05$ ) across the treatments in all the parameters evaluated. The initial body weight, final body weight, total weight, daily feed intake, total feed intake and feed conversion ratio had no significance effects as compared to the control (treatment A). This result agreed with the findings of Berry *et al.*, (2003) in which no significant difference

( $P>0.05$ ) was recorded in total weight gain, total feed intake feed conversion ratio among birds fed on single diet system. In other words, higher levels of fishmeal inclusion above 1.5 % progressively decrease the weight gain and/or performance of chickens. The result obtained in this study seems to suggest that both starter and finisher birds can be fed diets containing up to 1.5 % level of fishmeal using single diet without any observed weight depression. Higher levels of feed intake were however discovered among birds fed diet B (1.5 %). This might have been responsible for the differences in total weights recorded among the chickens. No mortality was recorded throughout the study period.

Table 3 shows the Carcass characteristics of broilers on single phase feeding system fed from 0 to 8 weeks. The results showed that all carcass parameters were not significantly affected ( $P>0.05$ ) by the dietary

**Table 2:** Growth performance of broiler birds fed 0 to 8 weeks on single phase feeding system.

Parameters	Inclusion levels of fishmeal (%)				SEM	LS
	A (0)	B (1.5)	C (2.5)	D (3.5)		
Initial body weight (g/b)	134.98	136.75	135.30	132.70	2.29	NS
Final body weight (g/b)	1,831.30	2,287.50	2,093.80	1,825.00	153.90	NS
Total weight gain (g/b)	1,696.30	2,150.80	1,958.50	1,692.30	181.05	NS
Daily weight gain (g/b/d)	26.16	34.58	30.51	27.88	2.88	NS
Total Feed Consumed(g/b)	3,556.80	4,141.50	3,757.80	3,912.50	181.15	NS
Daily Feed Intake (g/b/d)	70.18	66.13	72.58	65.71	4.01	NS
Feed Conversion Ratio	3.30	2.54	2.92	2.80	0.24	NS
Mortality	0.00	0.00	0.00	0.00	-	

NS = Not Significant, SEM = Standard Error Mean

**Table 3:** Carcass characteristics of broilers on single phase feeding system fed from 0 to 8 weeks.

Parameters	Dietary levels of fishmeal (%)				SEM	LS
	A (0)	B (1.5)	C (2.5)	D (3.5)		
Live weight (kg)	1.83	2.29	2.09	1.83	0.14	NS
Plucked weight (kg)	1.73	2.08	1.98	1.74	0.13	NS
Eviscerated weight (kg)	1.40	1.73	1.62	1.53	0.13	NS
Carcass weight (kg)	1.26	1.63	1.43	1.28	2.32	NS
Dressing weight %	68.60	71.86	67.66	69.64	7.20	NS
Head (g/bird)	53.00	58.31	53.13	49.75	4.57	NS
Leg (g/bird)	97.50	84.88	82.38	75.00	8.16	NS
Abdominal fat (g/bird)	22.63	44.13	43.13	47.75	2.53	NS
Gizzard (g/bird)	39.50	46.13	42.50	38.63	3.88	NS
Liver weight (g/bird)	46.50	53.88	50.00	42.63	0.69	NS
Kidney (g/bird)	4.38 <sup>b</sup>	7.13 <sup>a</sup>	4.50 <sup>b</sup>	4.89 <sup>c</sup>	0.70	*
Pancreas (g/bird)	6.38	5.75	6.00	6.13	0.47	NS
Spleen (g/bird)	4.38	3.75	4.13	3.38	1.09	NS
Lung (g/bird)	13.00	15.88	13.13	11.50	1.20	NS
Heart (g/bird)	11.38	12.63	12.13	13.25	1.20	NS
Caeca (g/bird)	16.13	18.25	13.75	15.63	1.97	NS
Small (g/bird)	94.25	113.75	98.25	100.12	8.32	NS
Large (g/bird)	5.50	5.13	6.13	6.50	1.03	NS

\* Significantly different ( $P < 0.05$ ), NS = Not Significant, SEM = Standard Error Mean

<sup>a, b, c</sup> = means within the same row having different superscripts are significantly different ( $p < 0.05$ )

treatments except for the kidney. However, kidney of birds fed diet B (1.5 %) fishmeal inclusion level was significantly ( $P > 0.05$ ) higher than that of the birds on other treatments. This may be as a result of the high nutritional value of the diet and consequence metabolic reaction

of enzymes. Dressing percentages was also found to be not significantly ( $P > 0.05$ ) different from the control diet. Eviscerated weight among chickens fed diets A, B, C and D were not significantly different, however, diet B has the highest mean (1.73 kg). The linear increase

in the weights of the intestine across the treatments may be due to their involvement in the digestion process. No differences ( $P>0.05$ ) were observed for abdominal fat deposition in the diets, but a lower mean was seen in the diet A (control).

### Impact

The use of single phase feeding in broiler programs might just be a solution to the ever multifaceted problem of feed cost in chicken production. It is simpler and can be adopted and practiced by laymen who cannot afford commercially manufactured feed for their birds. However, a standardised diet formula needs to be designed, tested and broadcasted in furtherance of the aforementioned objective.

### Conclusion

From the results obtained, it can be concluded that the use of fishmeal in a Single phase feeding system in broiler chickens has little significant effects in both performance and carcass characteristics of the birds. It can also be concluded that the birds fed diet B (1.5 %) level of fishmeal (21.05 % CP, 2900 ME) performed relatively better in terms of average body weight gain (2287.50 g) and average feed intake (4141.50 g) compared to birds fed diet C and D with 2.5 and 3.5 % inclusion levels respectively. It is therefore recommended that the inclusion of fishmeal in diets of broiler chickens using a single feeding phase should be not exceed 1.5 % for optimum output and efficient use of resources.

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## DETERMINATION OF CYANIDE CONCENTRATION IN BLOOD AND HISTOPATHOLOGICAL EFFECT OF CASSAVA WASTE-BASED FEEDING ON GOATS

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### Abstract.

Cassava (*Manihot esculenta*) is a major staple food for both humans and animals and feeding goats with its waste is common practice in southwest Nigeria. However, it contains endogenous cyanogenic glycosides that are readily hydrolyzed to liberate hydrogen cyanide that is poisonous. This study aimed to evaluate the effect of cassava waste (peels and leaves) on the kidney and liver of goats fed different inclusion level and determine the cyanide concentration in the blood of the goats. Ten West African dwarf male goats were divided into five groups and fed four diets of cassava waste ad-libitum for 16 weeks. Diet 1; 70% peels and 10% leaves, Diet 2; 50% peels and 20% leaves, Diet 3; 30% peels and 30% leaves, Diet 4; 10% peels and 40% leaves while the control were fed grass only. Blood samples were collected before the onset of the feeding, at the 8th week and 16th week. There were no clinical manifestation of cyanide poisoning in the animals and the Analysis of Variance (ANOVA) shows that haematological and biochemical parameters were not significantly different ( $p < 0.05$ ) across the 4 diets. High concentration of cyanide (80 ppm) was observed in the blood of animals fed Diet 4. Also the histopathological lesions of liver and kidney were more severe in animals fed Diet 4. The study therefore concludes that the high concentration of cyanide and the severity of the *histopathology* finding in Diet 4 can be attributed to the higher level of cassava leaves in the diet and hence, the leaves should be further processed before been feeding to goats.

**Keywords:** Cassava waste, Cyanide, Histopathology, Liver, Kidney, Goats.

## DETERMINATION DU TAUX DE CYANURE DANS LE SANG ET EFFET HISTOPATHOLOGIQUE DE L'ALIMENTATION A BASE DE DECHETS DE MANIOC SUR LES CAPRINS

### Résumé

Le manioc (*Manihot esculenta*) est un aliment de base à la fois pour les humains et les animaux, et l'alimentation des chèvres avec les déchets de cette plante est une pratique courante dans le sud-ouest du Nigeria. Cependant, le manioc contient des glycosides cyanogénétiques endogènes qui sont facilement hydrolysés pour libérer du cyanure d'hydrogène qui est toxique. Cette étude a pour objectif d'évaluer l'effet des déchets de manioc (pelures et feuilles) sur les reins et le foie des chèvres nourries à différents niveaux d'inclusion et de déterminer le taux de cyanure dans le sang des chèvres. Dix chèvres naines d'Afrique de l'Ouest ont été divisées en cinq groupes et nourries ad libitum pendant 16 semaines avec quatre régimes contenant des déchets de manioc. Le Régime 1 comportait 70% de pelures et 10% de feuilles ; le Régime 2 était constitué de 50% de pelures et 20% de feuilles ; le Régime 3 était composé de 30% de pelures et 30% de feuilles ; le Régime 4 comportait 10% de pelures et 40% de feuilles ; tandis que le régime témoin n'était constitué que d'herbe. Des échantillons de sang ont été prélevés avant le début de l'administration des régimes, à la 8ème semaine et à la 16ème semaine. On n'a noté aucune manifestation clinique d'intoxication au cyanure chez les animaux et l'Analyse de Variance (ANOVA) a montré que les paramètres hématologiques et biochimiques n'étaient pas significativement différents ( $p < 0,05$ ) dans les 4 régimes. Un taux élevé de cyanure (80 ppm) a été détecté dans le sang des animaux soumis au Régime

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4. Les lésions histopathologiques du foie et des reins étaient plus sévères chez les animaux nourris au Régime 4. L'étude conclut donc que le taux élevé de cyanure et la sévérité du résultat histopathologique dans le régime 4 peut être attribué à une importante inclusion de feuilles de manioc dans le régime et, par conséquent, les feuilles doivent être traitées avant d'être données aux chèvres comme aliments.

**Mots-clés :** déchets de manioc, Cyanure, Histopathologie, foie, rein, caprin.

## Introduction

Cassava (*Manihot esculenta*, Crantz) is emerging as a dominant staple food of primary or secondary importance in many developing countries of the humid and sub-humid tropics in Africa and elsewhere. It is the third most important food source in the tropics and is the staple food of tropical Africa with Nigeria being the current largest producer with an annual production estimated at 54 million metric tons (Akinpelu et.al. 2011, FAO 2005, FAOSTAT 2012). The crop is drought resistant, hence it is sometimes a nutritionally strategic famine reserve crop in areas of unreliable rainfall.

In spite of the important agricultural and nutritional roles played by cassava, its food value is greatly compromised by the presence of endogenous cyanogenic glycosides; especially linamarin and lotaustralin which under several prevailing tropical conditions are readily hydrolyzed to liberate hydrogen cyanide (Reddy, 2006). According to Liancheng et.al (1995) and Okigbo (1980), cassava contains 93% and 7% of linamarin and lotaustralin, respectively and their amounts vary with the part of the plant, its age, variety and environmental condition such as soil, moisture and temperature. All cassava organs, except seeds, contain cyanogenic glycosides (CG). Cassava with < 100mg kg<sup>-1</sup> and 100 – 500 mg kg<sup>-1</sup> CG content are called sweet and bitter cassava, respectively (Wheatley et. al., 1993). A lot of research had therefore been carried out by breeders and geneticists to develop varieties of cassava that have reduced level of cyanogenic glycosides (Siritunga and Sayre, 2004 & 2007).

Cassava (*Manihot esculenta* Crantz) is used by livestock farmers in the feeding of their animals. This, as posited by Okorie et.al., (2011), can be adduced to the competition for

other feed ingredients especially grains that are essentially the same as those required by humans and industries, nonetheless, its use in livestock feeding is only 5% according to Apata and Babalola (2012). The large amount of cyanogen which causes acute cyanide poisoning is a factor that limits its use. Cardoso et al., (2005) and Siritunga et al., (2004) in separate studies reported that linamarin is present in large amounts in the leaves and the peel of cassava roots (900–2000mg HCN in kg / fresh weight) and the leaves also contain a second enzyme called hydroxynitrile lyase, which catalyses the hydrolysis of acetone cyanohydrin to produce hydrogen cyanide and acetone. Unfortunately, these parts of the cassava plant are the choice of feed used for feeding animals.

In processing cassava, different methods had been used to reduce the level of these toxins (cyanogenic glycosides) but this is usually done for cassava that is processed into food for human consumption. Cassava fed to animals is either in form of waste such as peels from processing procedures or unprocessed roots. Farmers involved in rearing of goat are particularly concerned with feeding of their animals during the dry season when there is scarcity of grass for grazing hence they readily supplement their feeds with cassava wastes either processed or not.

In recent times, there is increase in the cultivation of cassava due to increased interest of the Nigerian government in cassava production and use in both human and animal feed which has led to its massive incorporation in livestock feeding.

This study therefore aimed to evaluate the effect of cassava waste (peels and leaves) on the kidney and liver of goats fed different inclusion levels and determine the residual cyanide concentration in the blood of the goats. It also tests the hypothesis that the

haematology and biochemical parameters of goats fed different inclusion levels of cassava waste-based feed are not significantly different.

## Materials and Methods

Ten healthy West African Dwarf goats, bucks, weighing between 6-8kg were obtained from reputable sources and housed intensively in a well-ventilated individual pens, measuring 1.2x0.75x1.8 m with asbestos roof and a wooden floor. Prior to arrival, each pen was disinfected with Izal® solution. On arrival, the goats were quarantined for a period of two weeks during which they were vaccinated against Peste des petit de ruminant (PPR), dewormed and treated against ecto-parasites with 0.2 ml/10 kg body weight of Ivomec®. The animals were fed elephant grass and were gradually introduced to concentrate diets that do not contain the test ingredients. Fresh water was supplied ad libitum. After the quarantine period, the animals were divided into five treatment groups in a Completely Randomized design at the rate of 2 animals per diet group. Each animal was kept and fed in a separate pen during the entire experimental period of 120 days.

Four different diets were compounded as shown in Table 1 on the basis of different percentage inclusion of dried cassava waste, 80% (Diet 1), 70% (Diet 2), 60% (Diet 3), 50% (Diet 4) and the control which were fed only grass. 10ml of blood per experimental animal were collected via venipuncture for determination of residual cyanide level, haematology and biochemical parameters. The collection of blood was carried out three times; before the commencement of the feeding trial, 8th week of the feeding trial and at the end, 16th week. Haematology and serum biochemical test were conducted on the blood samples to determine their deviation from normal using the Veterinary Merck's Manual as reference for normal range.

### Haematology Parameters Determination

Packed cell volume (PCV) was determined by filling a plain capillary tube with

blood to about three quarter length of the tube and the vacant end of the tube was sealed using plasticine. The sealed tubes were then centrifuged at a revolution of 12,000/minute for five minutes. Each tube was placed in micro haematocrit reader and the packed cell volume value was obtained and expressed in percentages. The haemoglobin (HB) concentration was determined spectrophotometrically according to the method of Franco 1984 as described in Cypress diagnosis kit. The Red blood cell (RBC) values were determined by use of the haemocytometer. Blood was diluted in ratio 1:200 with red blood diluting fluid using the red blood cell pipette. The dilution was mixed and left for 3 minutes after which the counting chamber of haemocytometer was charged and the red blood cell was counted using the ×40 objective of a microscope. The total number of cells counted was multiplied by 10,000 and expressed in cubic millimetre (mm<sup>3</sup>) or litre. Total white blood cell (WBC) was determined using the white blood cell pipette of the haemocytometer, blood was diluted in ratio 1:20 with white blood cell diluting fluid. The solution was gently mixed together and the counting chamber of the haemocytometer was charged with the solution and the total white blood cell was counted using the ×10 objective of the microscope. The total number of cell counted was multiplied by 50 and expressed in mm<sup>3</sup> or litre. White blood cell differentials were carried out by making a thin film of blood on a clean grease-free slide using a smooth edged spreader. The blood film was fixed with absolute methyl-alcohol for 3 – 5mins and allowed to dry. The film was stained with Giemsa stain and 100 white blood cell were differentiated using the oil immersion objective of microscope.

### Determination of biochemical parameters

#### Total protein (TP):

The concentration was determined spectrophotometrically according to the method of Tietz (1995) as described in Randox® kit total protein manual.

**Serum Albumin:**

The concentration was determined spectrophotometrically according to the method of Tietz (1995) as described in Randox® kit total protein manual.

**Globulin:**

the value for globulin was obtained from the difference between the total protein and the albumin.

**Serum glucose:**

The concentration was determined spectrophotometrically according to the method of Barham et al. (1972) as described in Randox® kit manual.

**Creatinine:**

Serum creatinine was determined spectrophotometrically according to the procedure of Henry (1974) as described in Randox® diagnostic kit.

**Aspartate aminotransferase (AST):**

This was determined spectrophotometrically according to the method of Reitman and Frankel (1957) as described in Randox® diagnostic kit.

**Alanine aminotransferase (ALT):**

This was determined spectrophotometrically according to the method of Reitman and Frankel (1957) as described in Randox® diagnostic kit.

For *histopathology*, one animal from each treatment was sacrificed and tissue samples from the liver and kidney were collected, fixed and store in 10% buffered formalin. Paraffin-embedded sections were stained with hematoxylin and eosin (H&E).

Determination of residual cyanide level in the blood was carried out using the procedure developed by Per Lundquist and Bo Sorbo (1998). The mean of the readings were obtained and the cyanide concentration was read from a standard graph, prepared with data for known amounts of 1 mmol/L KCN standard added to erythrocytes. The standard curve was

drawn using 0, 25, 50, 75, and 100µl of final concentrations of cyanide and the quantity of residual cyanide was determined by reading off the absorbance values obtained in the analysis of the sampled blood of the animals given different inclusion levels of cassava – waste

**Statistical analysis**

Data analysis was performed using statistical software (SPSS 16). Analysis of Variance (ANOVA) was used to test the differences in both the *haematology* and biochemical parameters and the different means were separated using Duncan's Multiple Range test. The level of significance was set at  $p < 0.05$ .

**Results****Haematology and biochemical parameters**

The clinical examination of the animals indicated that there were no clinical manifestations of cyanide intoxication during the study. This can be because of the sulphur incorporated into the different diets (Table 1).

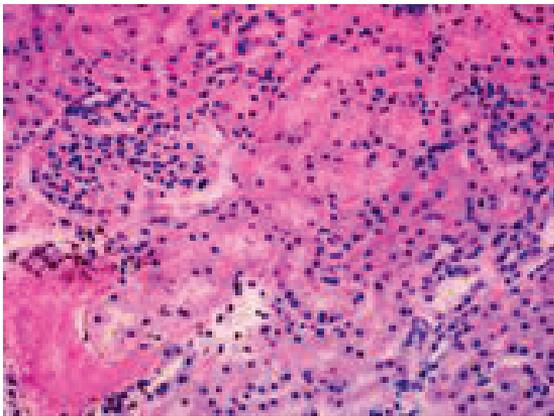
Biochemical and *haematology* results are presented in Table 2. The result shows that there is no significant difference ( $p < 0.05$ ) in the haematological parameters of the goats fed different inclusion level of cassava waste-based feed when the diets were compared with each other. Of the biochemical parameters taken, alanine transaminase (ALT = 31.45) is significantly different ( $p < 0.005$ ) in Diet 3 from all the other diets, however, this value still falls within the Veterinary Merck's normal range for goats (Table 2)

**Determination of cyanide concentration in blood of experimental animals.**

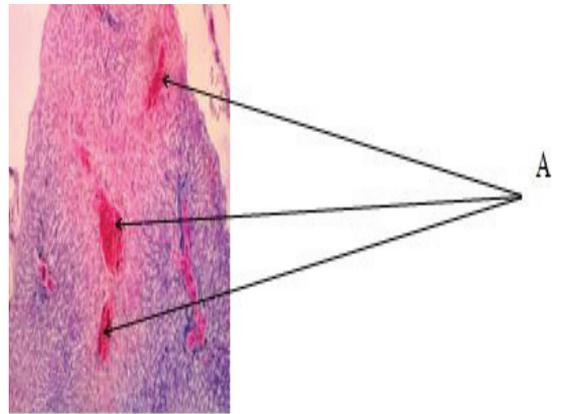
Blood samples were collected from the animals for determination of the presence and level of residual cyanide. From the result obtained, as presented in Table 3, it was observed that there was increase in the level of residual cyanide in the blood of all the animals fed the different diets as the project progressed. The values obtained were checked off on the graph of the standard curve to determine the

**Table 1:** Feed Composition showing the percentage inclusion of dried cassava peels and leaves.

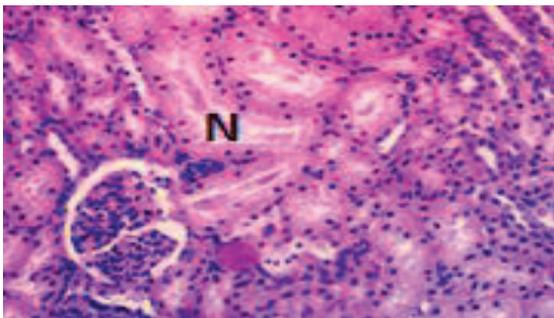
Ingredient	Diet 1 (80%)	Diet 2 (70%)	Diet 3 (60%)	Diet 4 (50%)	Control
Cassava peels	700	500	300	100	Only Grass.
Cassava leaves	100	200	300	400	
Cowpea haulms	175	275	375	475	
Bone Meal	10	10	10	10	
Sulphur	10	10	10	10	
Salt	5	5	5	5	
<b>Total</b>	<b>1000</b>	<b>1000</b>	<b>1000</b>	<b>1000</b>	



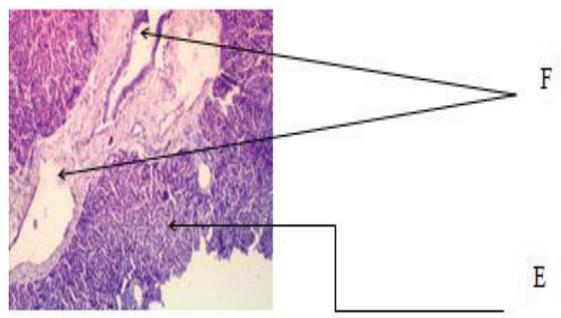
**Figure 1:** Section of the kidney of goat fed 80% inclusion of cassava waste (Diet 1) showing severe diffuse tubular necrosis and degeneration in the kidney. X400 H&E



**Figure 2:** Section of the liver of goat fed 80% inclusion of cassava waste (Diet 1) showing severe diffuse centrilobular areas of necrosis while the blood vessels are severely congested.(A) X100 H&E



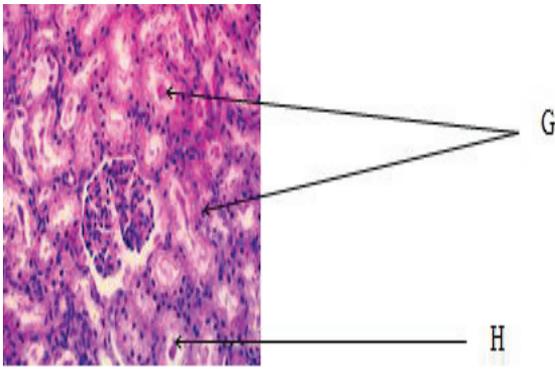
**Figure 3:** Section of the kidney of goat fed 70% inclusion of cassava waste (Diet 2) showing severe diffuse tubular necrosis and degeneration in the kidney. X350, H&E



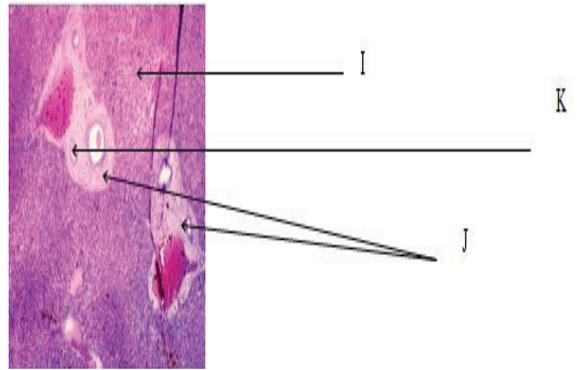
**Figure 4:** Section of the liver of goat fed 70% inclusion of cassava waste (Diet 2) showing E = severe diffused peri-portal fibrosis. In addition to that, there is diffused hepatic atrophy and sinusoidal dilatation (F). X100, H&E

concentration. The values obtained shows that the safe level of 10 ppm ( $\mu\text{l}$ ) recommended by WHO, (FAO/WHO, 1991) was only observed at the beginning of the experiment in all the

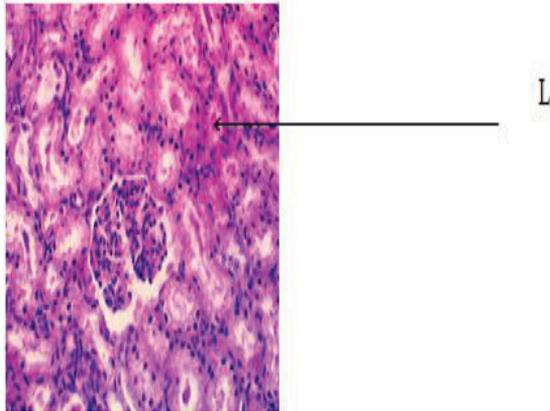
experimental groups and throughout for the control group that was fed grass only. At the 8th week, Diets 1, 2 and 3 had 13.75ppm, 16.25ppm and 28.75ppm respectively while



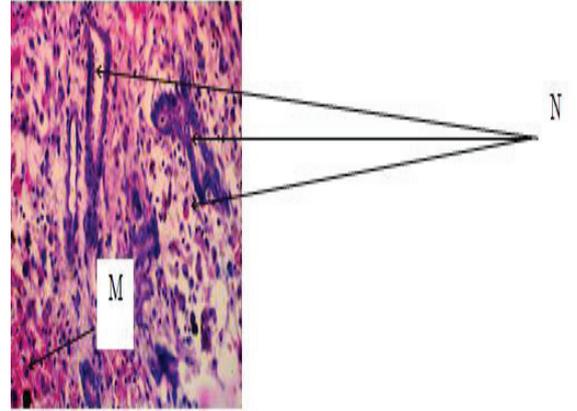
**Figure 5:** Section of the kidney of goat fed 60% inclusion of cassava waste (Diet 3) show: G = severe diffused tubular necrosis and degeneration in the kidney. H= dilated tubular lumen containing protein casts.X400, H&E



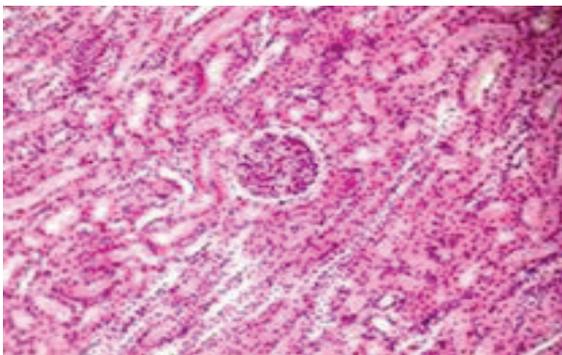
**Figure 6:** Section of the liver of goat fed 60% inclusion of cassava waste (Diet 3) showing I = severe diffuse periportal fibrosis but with (J) mild centrilobular area of necrosis. There is also proliferation, of the bile duct (K ).X100H&E



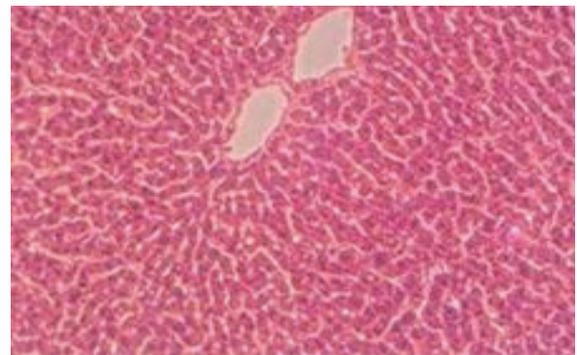
**Figure 7:** Section of the kidney of goat fed 50% inclusion of cassava waste (Diet 4) showing severe diffused tubular necrosis and degeneration in addition to dilated .tubular lumen which contains protein casts.X400H&E



**Figure 8:** Section of the liver of goat fed 50% inclusion of cassava waste (Diet 4) showing severe M= peri-portal necrosis with hemorrhages in the liver. N = diffuse bile duct proliferation with severe erythrophagocytosis by kupffer cells.X400H&E



**Figure 9:** Section of the kidney of goat fed grass alone showing no pathological lesions.X 200



**Figure 10:** Section of the liver of goat fed grass alone showing no pathological lesions. X 300, H&E stain

Diet4 recorded 80.00ppm. There was a slight increase in the values of Diets 1, 2 and 3 at the 16th week (31.25ppm 30.00ppm and 31.25ppm respectively) however Diet 4 maintained the 80.00ppm value at the 16th week. Diet 4 is of particular interest considering the high level of residual cyanide detected in the blood consistently throughout the life of the experiment.

In this study, Diet 4 has a consistent concentration of 80ppm, it is however within the moderately poisonous level.

*Histopathological* findings in the tissues of animals fed different inclusion levels of cassava-waste feed

*Histopathology* evaluation was carried out on the sacrificed animals at the end of the project life. No gross lesions were found in any of the animals, however, on histopathological evaluation, there was no histopathological lesions seen in the animal on Diet 5 (grass only) while goats fed the different inclusions of cassava waste presented lesions.

In the kidney samples collected from animals on Diet 1 (80%), Diet 2 (70%) and Diet 3 (60%), there is severe diffuse tubular necrosis and degeneration while the tubular lumen of Diet 3 contains protein casts and the tubules were dilated in addition (Figures 1,3,5). The levels of severity in lesions of the kidney of animal on Diet 4 (50%) were higher than in the

**Table 2:** Haematology and serum parameters of the goats fed different inclusion level of cassava waste-based feed

Parameters	Diets					SEM	Merck's manual Values
	Control (grass only)	Diet 1 (80%)	Diet 2 (70%)	Diet 3 (60%)	Diet 4 (50%)		
PCV (%)	20.67	26.67	26.34	22.89	24.78	± 2.37	22 – 38
RBC (x 106/ $\mu$ l)	7.55	9.71	9.55	8.24	9.44	± 0.85	8 – 18
WBC( x 103/ $\mu$ l)	6.42	5.06	5.12	5.26	5.89	± 0.69	4 – 13
HB (g/dl)	7.28	8.79	9.07	7.81	8.58	± 0.68	8 – 15
MCV (fl)	28.85	27.47	27.81	27.81	28.70	± 0.59	16 – 25
TP (g/dl)	6.15	6.01	5.77	5.12	5.51	± 0.33	6.1– 7.5
Urea (mg/dl)	15.12	16.77	16.72	16.63	16.02	± 2.18	13 – 26
Creatinine (mg/dl)	1.18	0.86	1.28	1.03	1.30	± 0.38	0.7– 1.50
AST (u/l)	50.67	52.56	58.78	67.67	62.67	± 6.95	66– 230
ALT (u/l)	22.78 <sup>a</sup>	21.22 <sup>a</sup>	24.78 <sup>a</sup>	31.45 <sup>b</sup>	22.78 <sup>a</sup>	± 3.43	15 – 52

<sup>a,b</sup> Mean value with different superscripts for each parameter differ significantly ( $P<0.05$ )

Source: Laboratory analysis, 2014

**Table 3:** Concentration of cyanide in blood of experimental animals

	ABSORBANCE			CONCENTRATION $\mu$ L (PPM)		
	1st week	8th week	16th week	1st week	8th week	16th week
Control	0.244	0.276	0.288	0.00	6.25	7.25
Diet 1 (80%)	0.239	0.344	0.489	0.00	13.75	31.25
Diet 2 (70%)	0.286	0.357	0.479	7.50	16.25	30.00
Diet 3 (60%)	0.298	0.463	0.488	7.50	28.75	31.25
Diet 4 (50%)	0.282	0.834	0.833	6.25	80.00	80.00

Source: Laboratory analysis, 2014

other groups. It shows severe diffused tubular necrosis and degeneration in addition to the tubular lumen which contains protein casts and the tubules were also dilated (Figure 7).

The liver sample of animals on Diet 1 (80%) and Diet 2 (70%) shows severe diffuse periportal fibrosis and there are centrilobular areas of necrosis while the blood vessels are severely congested (Figures 2 & 4). In addition to that, Diet 2 animal had diffused hepatic atrophy and sinusoidal dilatation in the liver (Figure 4). The liver samples of animal on Diet 3 (60%) also shows severe diffuse periportal fibrosis but with mild centrilobular areas of necrosis. There is also proliferation, hyperplasia, of the bile duct (Figure 6). The liver sample of Diet 4 animal had severe centrilobular necrosis with hemorrhages in the liver and there is also diffuse bile duct proliferation with severe erythrophagocytosis by kupffer cells (Figure 8).

## Discussion

The lack of clinical manifestation of cyanide intoxication during the study is attributed to the presence of sulphur in the diets and this is supported by Merck's Manual (2012) and Smith (1988) that there is evidence that ruminants can use a variety of sulphur donors including elemental sulphur to detoxify cyanide of dietary origin and it is rapidly detoxified in the rumen and liver. Biochemical profile of any species of animals, when combined with *haematology* forms the basis for the diagnosis of most disease condition and physiological state. Though many of the biochemical parameters tend to have specificity for an organ, the interpretation requires an understanding of the pathological implications of each abnormal result. Together with the normal reference results, these form a pattern which reflects one or more underlying disease process. In the present study the value of Alanine transaminase (ALT) is significantly different in Diet 3 it is however still within the normal range for goats hence it can be concluded that the diet does not adversely affect the animals in the group.

The high level of residual cyanide

(80.00ppm) detected all through the life of the study in Diet 4 can be adduced to the fact that linamarin is present in large amounts in the leaves and the peel of cassava roots (900–2000mg HCN / kg fresh weight) and the leaves also contain a second enzyme called hydroxynitrile lyase, which catalyses the hydrolysis of acetone cyanohydrin to produce hydrogen cyanide and acetone (Cardoso *et al.*, 2005 and Siritunga *et al.*, 2004). Diet 4 contains a higher proportion of cassava leaves, 40%. According to Merck's Veterinary Manual (2012), forage containing <100 ppm HCN, wet weight, is usually safe to pasture. However Cardoso *et al.* (2005) is of the opinion that useful guide based on total root cyanide content of < 50ppm is innocuous, 50 -100ppm is moderately poisonous and >100 ppm dangerously poisonous.

The pathological lesions seen in the animals fed the different inclusions of cassava waste are consistent with lesions of cyanide poisoning. Though the animals did not show any clinical presentation of poisoning during the period they were fed, the lesions in the tissues however show the effect of the feed on the organs, the liver and the kidney. These organs are primarily responsible for detoxification and removal of cyanide in the body.

The findings of this study support the studies of Shivanoor and David (2014), Soto-Blanco and Gorniak (2010), Soto-Blanco *et al.*, (2005) and Sousa *et al.*, (2002) that established that cyanide causes hepatotoxicity and nephrotoxicity in animals. However, the level of severity discovered in this present study differs from those other studies and could be attributed to the combination of both the cassava peels and cassava leaves in the feed. The level of severity of the lesions in animal from Diet 4 support the finding of higher concentration of cyanide in the blood as presented in Table 3.

## Conclusion

From the findings in this study, it can be concluded that the additional enzyme in cassava leaves, hydroxynitrile lyase is the cause of the increased level of cyanide in the blood of goats

fed Diet 4 (40% cassava leaves, 10% cassava peels) and could also be the reason for the more severe lesions seen in the *histopathology*. Hence the leaves need to be further processed or the level reduced in future composition of the feed. Inclusion of cassava leaves in the diets of goats should be further researched into with the aim of eliminating or reducing the level of cyanide therein. This is suggested so that the protein content of the leaves can be taken advantage of in the ration/feed composition for goats.

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## VITAMINS AND ADMINISTRATION PERIODS EFFECTS ON HAEMATOLOGICAL PARAMETERS OF BROILER CHICKENS VACCINATED AGAINST NEWCASTLE DISEASE

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### Abstract

A total of two hundred and seventy (270) Arbor acre day old chicks were used to investigate the effects of vitamins and administration periods on haematological parameters of broiler chickens vaccinated against Newcastle disease. The experiment lasted for a period of 8 weeks. Birds were brooded together at day old and on day 2 of age, the 270 broiler chicks were divided based on weight equalization into nine (9) treatment groups, replicated thrice with ten birds per replicate. The 9 treatments consist of a factorial arrangement of 4 dietary supplemental vitamins and two administration periods with a control. The birds were managed intensively, Newcastle disease vaccines were administered on the 5th (Hitchner B1) and 24th (Lasota) days of age. Data obtained on haematological indices were subjected to Analysis of Variance in a completely randomized design. Significantly ( $p < 0.05$ ) higher Mean corpuscular volume and Mean corpuscular haemoglobin (146.31 fl and 48.96 pg) were recorded in birds fed diet supplemented with vitamin C at 14th day post-Hitchner B1. Dietary supplementation with vitamins A three days pre-Lasota resulted to higher Haemoglobin (9.70 g/dl) and Mean corpuscular haemoglobin concentration (35.28 g/dl) at 14th day post-Lasota. In conclusion, vitamins C (50 mg/kg) dietary supplementation is recommended for improved haematological indices (MCV and MCH) up to the 14th day post Hitchner B1 vaccine, while administration of vitamin A (9.1 mg/kg) supplemented diet three days pre-Lasota can be used to enhance better Haemoglobin and Mean corpuscular haemoglobin concentration at 14th day post-Lasota.

**Keywords:** Vitamins, Haematological parameters, Newcastle disease and Broiler chickens

## EFFETS DES VITAMINES ET DES PERIODES DE LEUR ADMINISTRATION SUR LES PARAMETRES HEMATOLOGIQUES DES POULETS DE CHAIR VACCINÉS CONTRE LA MALADIE DE NEWCASTLE

### Résumé

Un total de deux cent soixante-dix (270) poussins Arbor acre âgés d'un jour a été utilisé pour l'étude des effets des vitamines et des périodes de leur administration sur les paramètres hématologiques des poulets de chair vaccinés contre la maladie de Newcastle. L'expérience a duré 8 semaines. Les oiseaux ont été incubés ensemble aux 1<sup>er</sup> et 2<sup>ème</sup> jours d'âge ; les 270 poulets de chair ont été répartis sur base de l'égalisation du poids en neuf (9) groupes de traitement, répétés trois fois avec dix oiseaux par répétition. Les 9 traitements consistaient en un arrangement factoriel de 4 compléments alimentaires avec vitamines et deux périodes d'administration, avec un témoin. Les oiseaux ont été élevés de manière intensive ; les vaccins contre la maladie de Newcastle ont été administrés au 5<sup>ème</sup> jour (Hitchner B1) et au 24<sup>ème</sup> jour (Lasota) d'âge. Les données obtenues sur les indices hématologiques ont été soumises à l'analyse de variance dans un schéma complètement aléatoire. Un volume corpusculaire moyen significativement ( $p < 0,05$ ) élevé et une hémoglobine corpusculaire moyenne élevée (146,31 et 48,96 pg) ont été enregistrés chez les oiseaux recevant de la vitamine C au 14<sup>ème</sup> jour post-Hitchner B1. Un complément alimentaire en vitamines A trois jours pré-Lasota a entraîné une augmentation du taux d'hémoglobine (9,70 g / dl) et de la concentration moyenne d'hémoglobine corpusculaire (35,28 g / dl) au 14<sup>ème</sup> jour post-

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Lasota. En conclusion, la supplémentation alimentaire en vitamines C (50 mg / kg) est recommandée pour l'amélioration des indices hématologiques (MCV et MCH) jusqu'au 14ème jour après le vaccin Hitchner BI, tandis que l'administration du régime supplémenté en vitamine A (9.1mg / kg) trois jours pré-Lasota peut être utilisée pour un meilleur taux d'hémoglobine et d'hémoglobine corpusculaire moyenne au 14ème jour post-Lasota.

**Mots-clés :** vitamines, paramètres hématologiques, maladie de Newcastle et poulets de chair

## Introduction

Haematological indices are good indicators of the health, physiological status of animals, and are of ecological significance in understanding the connections between blood characteristics and the environment (Ovuru and Ekweozor, 2004).

Broiler chickens compared to other domestic animals are more susceptible to stressors from changing environmental conditions (Nolan *et al.*, 1999). Increased environmental temperature reduces feed intake, growth rate, feed utilization, dressing yield and affect the health status leading to increased mortality rates in broiler chickens (Geraert, 1998). This is an addition to considerable level of stress resulting from vaccination. A possible approach to reducing the negative impact of environmentally imposed stressors is the roles of vitamins at levels beyond dietary recommendations for deficiency symptoms. It is also worthy of note that many vitamins may be lost during feed processing and storage, hence some amount of vitamins added to feed by commercial feed millers might be unavailable to birds. Several studies had considered the influence of vitamins and feeding manipulations on the productive performance in poultry. Sahina *et al.* (2003) and Sabah *et al.* (2008) reported that nutritional modifications can be used to influence productivity, health and physiological processes occurring in broilers reared in high ambient temperatures. Micronutrients like vitamins A and C have been shown to play vital roles in host immune response (Karamouz *et al.*, 2010). In broiler chickens production in Nigeria, vaccination is usually undertaken as a reliable preventive measure against Newcastle disease, with oral administration of commercially available multi-

vitamins as adjunct to improve productivity. However, the use of the constituent vitamins of these multi-vitamins as dietary supplement pre- or post-vaccinations is not common and can be investigated. Therefore this study determined the effects of supplemental vitamins and periods of administration on haematological parameters of broiler chickens vaccinated against Newcastle disease.

## Materials and Methods

### *Experimental Site*

The field work was carried out at the Poultry unit of the Directorate of University Farms (DUFARMS), while the Laboratory aspects of the study were undertaken at the Anatomy and Physiology Laboratory, College of Veterinary Medicine, located within latitude 7° 13' N and longitude 3° 26' E (Google Earth, 2016), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

### *Experimental birds and management*

A total of two hundred and seventy (270) day old Arbor acre broiler chicks were sourced from a reliable hatchery in Abeokuta, Ogun State, Nigeria. These chicks were used for the study for a period of 8 weeks. The birds were intensively managed ( Deep-litter) through starter and finisher phases with the provision of a single straight diet containing 20.82 % crude protein, 3.90 % ether extract, 3.86 % crude fibre, 0.25 % lysine 0.25 % methionine, 12.16 MJ/kg Metabolizable Energy and water (ad-libitum).

### *Experimental procedures*

The two hundred and seventy (270) day old Arbor acre chicks were brooded together in a single brooding unit at day old

and at day 2 of age, the chicks were divided by weight balancing into nine (9) treatment groups each in three (3) replicates with ten (10) birds per replicate. The nine (9) treatment groups consists of a factorial arrangement of four (4) vitamins (A, C, E and combination of A, C, E) and two administration periods (3 days pre- and post-Newcastle disease vaccination) with a control. Birds were vaccinated with Newcastle disease vaccines on the 5th (Hitchner BI, intra-ocularly) and 24th (Lasota, orally) days of age and were fed a straight diet all through the period of the study with vitamins only administered in the diets at three days pre- and post-Newcastle disease vaccination as dietary supplement at the following rates of inclusions: vitamin A (0.45mg/kg diet), vitamin C (50mg/kg diet), vitamin E (9.1 mg/kg diet) and combined vitamins A+C+E (0.15+16.67+3.0 mg/kg diet). Blood samples were collected at the 19th and 38th days of age (14th day post-Newcastle disease vaccination) for evaluating haematological indices.

#### *Experimental design and treatment description*

The experiment is a 4x2 factorial layout consisting of four (4) vitamins and two (2) administration periods with a control in a Completely Randomized Design. The nine (9) treatment groups consist of the following: Control (no dietary vitamins supplementation); vitamin A (0.45 mg/kg diet) administered 3 days pre-Newcastle disease vaccination; vitamin A (0.45 mg/kg diet) administered 3 days post- Newcastle disease vaccination; vitamin C (50 mg/kg diet) administered 3 days pre- Newcastle disease vaccination; vitamin C (50 mg/kg diet) administered 3 days post-Newcastle disease vaccination; vitamins E (9.1 mg/kg diet) administered 3 days pre-Newcastle disease vaccination; vitamins E (9.1 mg/kg diet) administered 3 days post-Newcastle disease vaccination; combined vitamins A+C+E (0.15+16.67+3.03 mg/kg diet) administered 3 days pre- Newcastle disease vaccination and the combined vitamins A+C+E (0.15+16.67+3.03 mg/kg diet) administered 3 days post-Newcastle disease vaccination, respectively.

#### *Collection of blood samples for Haematology*

At the 19th and 38th days of age, three (3) birds were selected from each replicate and 2 ml of blood sample was collected from each bird using wing web vein puncture method into bottles containing Ethylene Diamine Tetra-Acetic acid (EDTA).

#### *Determination of Haematological parameters*

The White blood cell, Red blood cell, Haemoglobin, and Packed cell volume were determined according to the method of Dacie and Lewis (1995). The values obtained for Red blood cell, Haemoglobin, and Packed cell volume were used to calculate the Mean corpuscular volume, Mean corpuscular haemoglobin and Mean corpuscular haemoglobin concentration. The differential White blood cell counts were obtained by making a differential smear stained with wright's stain and the percentage counts taken for lymphocytes, heterophils, eosinophils, monocytes and basophils.

#### *Statistical Analysis*

Data obtained were arranged in a 4x2 factorial layout with a control and subjected to Analysis of Variance in Completely Randomized Design. Significantly ( $p < 0.05$ ) different means among variables were separated using Duncan's Multiple Range, F- Test as contained in SAS (2010) package.

## **Results**

#### *Effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post-Hitchner BI vaccine*

The main effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post-Hitchner BI vaccine are shown in Table I. Significantly ( $p < 0.05$ ) higher Mean corpuscular volume and Mean corpuscular haemoglobin values of 146.31 fl and 48.96 pg were obtained in birds fed diet supplemented with vitamin C, while lower Mean corpuscular volume (111.51, 114.51 and 119.49 fl) were recorded in birds fed the control diets, birds fed diet supplemented with vitamins E and

combined vitamins (A+C+E). Also, lower Mean corpuscular haemoglobin values of 37.26 and 38.62 pg were obtained in birds fed the control diet and those fed diet supplemented with vitamin E. The administration periods of the vitamins did not significantly ( $p > 0.05$ ) influence any of the haematological parameters. The interactive effects of supplemental vitamins and administration periods of vitamins at 14th day post-Hitchner BI is presented in Table 2. A significantly ( $p < 0.05$ ) higher Mean corpuscular volume of 159.39 fl was obtained in birds fed diet supplemented with vitamin C administered three days post-Hitchner BI. On the contrary, significantly ( $p < 0.05$ ) lower Mean corpuscular volumes of 111.51, 108.11, and 116.76 fl were recorded in birds fed the control diet, birds fed diet supplemented with vitamin E three days pre-Hitchner BI and those fed diet supplemented with combined vitamins (A+C+E) three days post-Hitchner BI.

*Effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post-Lasota vaccine.*

The main effects of vitamins and administration periods on the haematological parameters of broiler chickens at 14th day post-Lasota are shown in Table 3. Significantly ( $p < 0.05$ ) higher Haemoglobin and Mean corpuscular haemoglobin concentration (9.55 and 34.12 g/dl) were obtained in birds fed the control diet, while significantly ( $p < 0.05$ ) lower Haemoglobin and Mean corpuscular haemoglobin concentration (8.00 g/dl and 31.35 g/dl) were obtained in birds fed diet supplemented with the combined vitamins (A+C+E). The administration periods of vitamins did not significantly ( $p > 0.05$ ) affect any of the haematological parameters considered. Table 4 shows the interactive effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post-Lasota. The highest Haemoglobin (9.70 g/dl) were obtained in birds fed vitamin A supplemented diet administered three days pre-Lasota; and birds fed diet supplemented with vitamin C (9.60 g/dl) three days post-Lasota. However, the significantly ( $p < 0.05$ )

lowest haemoglobin (7.95 g/dl) was obtained in birds offered diet supplemented with combined vitamins (A+C+E) three days post-Lasota. Furthermore, significant ( $p < 0.05$ ) highest Mean corpuscular haemoglobin concentration (35.28 g/dl) was recorded in birds fed diet supplemented with vitamin A three days pre-Lasota, while the lowest significant ( $p < 0.05$ ) Mean corpuscular haemoglobin concentration (31.36 and 30.93 g/dl) were obtained in birds fed diet supplemented with vitamin A three days post-Lasota and birds offered diet supplemented with combined vitamins (A+C+E) three days pre-Lasota.

## Discussion

The significantly higher Mean corpuscular volume and Mean corpuscular haemoglobin values in birds fed diet supplemented with vitamin C at 14th day post-Hitchner BI administration indicated that vitamins C positively influenced the haematopoietic process. Also, the improved Haemoglobin (in birds fed vitamins A and C supplemented diets) and Mean corpuscular haemoglobin concentration in birds fed diet supplemented with vitamins A at 14th day post-Lasota also implies that vitamins A, and C dietary supplementation enhanced the Mean corpuscular volume, Mean corpuscular haemoglobin, Haemoglobin and Mean corpuscular haemoglobin concentration. This result is similar to the findings of Afolabi (2011) who concluded that the number of erythrocytes and other components of blood differed due to nutritional status. However, this is in variance with the report of Sanda and Oyewole (2015) who observed no differences in the haematological parameters of birds fed diet supplemented singly with vitamins A, C and combination of vitamins A and C. The lower Haemoglobin and Mean corpuscular haemoglobin concentration observed in birds fed diet supplemented with the combined vitamins indicated that the synergistic action of vitamins A, C and E did not enhanced the haematopoietic process at 14th day post-Lasota. This can be attributed to the varying

**Table 1:** Main effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post-Hitchner B1

Parameter	Vitamins				Periods of Administration		
	Control	A(0.45 mg/kg)	C(50mg/kg)	E(9.1mg/kg)	A+C+E (0.15+16.67 +3.03 mg/ kg)	PRV	PSV
Packed cell volume (%)	26.33±0.21	27.17±1.47	28.67±2.09	28.00±0.89	29.17±0.95	26.93±0.43	28.80±1.01
Haemoglobin (g/dl)	8.80±0.10	9.00±0.46	9.57±0.64	9.48±0.40	9.83±0.28	9.07±0.17	9.60±0.33
Red blood cell (x1018/µl)	2.37±0.06	2.25±0.24	2.07±0.29	2.55±0.26	2.45±0.11	2.29±0.10	2.38±0.16
Mean corpuscular volume (fl)	111.51±2.11 <sup>b</sup>	124.90±8.74 <sup>ab</sup>	146.31±13.15 <sup>a</sup>	114.51±10.03 <sup>b</sup>	119.49±2.26 <sup>b</sup>	119.91±4.59	126.77±7.10
Mean corpuscular haemoglobin (pg)	37.26±0.73 <sup>b</sup>	41.48±3.13 <sup>ab</sup>	48.96±4.46 <sup>a</sup>	38.62±3.18 <sup>b</sup>	40.33±0.97 <sup>ab</sup>	40.37±1.56	42.29±2.39
Mean corpuscular haemoglobin concentration (g/dl)	33.41±0.12	33.18±0.48	33.43±0.42	33.83±0.54	33.74±0.26	33.68±0.24	33.36±0.24
White blood cell (x1015/µl)	9.07±0.35	12.60±0.89	11.58±0.87	11.25±0.26	10.58±0.87	10.85±0.48	11.99±0.57
Heterophils (%)	29.00±1.10	28.33±1.38	30.33±1.69	31.00±1.59	30.50±1.48	28.80±1.02	30.87±0.69
Lymphocytes (%)	68.67±0.42	68.50±1.65	67.67±1.67	67.33±1.45	67.00±1.21	68.60±0.91	67.07±0.68
Heterophil/Lymphocyte	0.42±0.02	0.41±0.03	0.45±0.03	0.46±0.03	0.46±0.03	0.42±0.02	0.46±0.01
Eosinophils (%)	0.33±0.21	1.33±0.61	0.50±0.50	0.67±0.33	0.83±0.40	0.93±0.27	0.53±0.27
Basophils (%)	0.33±0.21	0.83±0.31	0.67±0.33	0.33±0.21	0.50±0.34	0.47±0.19	0.60±0.16
Monocytes (%)	1.67±0.56	1.00±0.37	0.83±0.31	0.67±0.33	1.17±0.54	1.20±0.28	0.93±0.27

<sup>ab</sup> Means on the same row having different superscript are significantly ( $p < 0.05$ ) different.  
 PRV: 3 days pre-Newcastle disease vaccination administration of vitamins; PSV: 3 days post-Newcastle disease vaccination administration of vitamins

**Table 2:** Interactive effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post- Hatcher BI

Parameters	Control	A (0.45 mg/kg)		C (50mg/kg)		E (9.1mg/kg)		A+C+E (0.15+16.67+3.03 mg/kg)	
		PRV	PSV	PRV	PSV	PRV	PSV	PRV	PSV
Packed cell volume (%)	26.33±0.33	26.67±1.45	27.67±2.91	26.33±0.33	31.00±4.04	27.33±0.88	28.67±1.67	28.00±1.53	30.33±0.88
Haemoglobin (g/dl)	8.80±0.15	8.73±0.58	9.27±0.80	8.97±0.24	10.17±1.29	9.43±0.41	9.53±0.80	9.43±0.43	10.23±0.23
Red blood cell (x10 <sup>18</sup> /µl)	2.367±0.08	2.20±0.31	2.30±0.44	2.03±0.24	2.10±0.60	2.57±0.26	2.53±0.52	2.30±0.17	2.60±0.10
Mean corpuscular volume (fl)	111.51±3.34 <sup>b</sup>	124.52±13.59 <sup>ab</sup>	125.28±14.05 <sup>5b</sup>	133.22±15.93 <sup>ab</sup>	159.39±20.96 <sup>a</sup>	108.11±8.12 <sup>b</sup>	120.92±19.95 <sup>ab</sup>	122.21±3.93 <sup>ab</sup>	116.76±1.69 <sup>b</sup>
Mean corpuscular haemoglobin (pg)	37.26±1.15	40.59±3.77	42.36±5.83	45.53±6.09	52.38±7.12	37.22±2.40	40.02±6.55	41.24±1.88	39.40±0.60
Mean corpuscular haemoglobin concentration (g/dl)	33.41±0.18	32.72±0.57	33.64±0.79	34.05±0.70	32.82±0.20	34.49±0.38	33.17±0.93	33.72±0.45	33.75±0.37
White blood cell (x10 <sup>15</sup> /µl)	9.07±0.35	12.58±1.89	13.07±1.69	10.47±0.57	12.70±1.47	10.90±0.32	11.60±0.32	11.20±1.47	9.97±1.13
Heterophils (%)	29.00±1.73	27.00±2.65	29.67±0.88	28.33±2.96	32.33±1.20	31.33±2.91	30.67±2.03	28.33±2.03	32.67±1.45
Lymphocytes (%)	68.67±0.66	70.00±2.65	67.00±2.08	68.67±3.18	66.67±1.67	66.33±2.73	68.33±1.45	69.33±0.67	64.67±1.20
Heterophil/Lymphocyte	0.42±0.02	0.39±0.05	0.44±0.03	0.41±0.06	0.48±0.03	0.47±0.06	0.45±0.04	0.41±0.03	0.51±0.03
Eosinophils (%)	0.33±0.33	1.00±0.58	1.67±1.20	1.00±1.00	0.00±0.00	1.00±0.58	0.33±0.33	1.33±0.67	0.33±0.33
Basophils (%)	0.33±0.33	1.00±0.58	0.67±0.33	0.67±0.67	0.67±0.33	0.33±0.33	0.33±0.33	0.00±0.00	1.00±0.58
Monocytes (%)	1.67±0.88	1.00±0.58	1.00±0.58	1.33±0.33	0.33±0.33	1.00±0.58	0.33±0.33	1.00±1.00	1.33±0.67

**Table 3:** Main effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post-Lasota

Parameter	Vitamins				Periods of Administration		
	Control	A(0.45 mg/kg)	C(50mg/kg)	E(9.1mg/kg)	A+C+E (0.15+16.67 +3.03 mg/ kg)	PRV	PSV
Packed cell volume (%)	28.00±0.58	27.00±0.91	27.25±1.75	26.75±0.48	25.50±0.65	26.7±0.62	27.10±0.64
Haemoglobin (g/dl)	9.55±0.14a	9.00±0.47ab	8.98±0.53ab	8.85±0.16ab	8.00±0.29b	8.85±0.27	8.90±0.26
Red blood cell (x1018/µl)	2.50±0.06	2.15±0.23	2.38±0.36	2.38±0.09	1.85±0.09	2.22±0.12	2.28±0.15
Mean corpuscular volume (fl)	112.34±4.90	128.85±10.26	119.98±11.97	113.04±3.70	138.83±7.88	122.82±5.45	122.40±6.26
Mean corpuscular haemoglobin (pg)	38.30±1.46	42.79±3.12	39.65±4.26	37.41±1.37	43.64±3.12	40.67±1.87	40.05±1.84
Mean corpuscular haemoglobin concentration (g/dl)	34.12±0.19 <sup>a</sup>	33.32±1.16 <sup>ab</sup>	32.99±0.69 <sup>ab</sup>	33.09±0.42 <sup>ab</sup>	31.35±0.50 <sup>b</sup>	33.13±0.55	32.82±0.42
White blood cell (x1015/µl)	9.70±0.40	11.83±1.55	12.03±0.48	11.86±1.24	10.48±0.62	11.81±0.67	10.55±0.53
Heterophils (%)	26.50±0.87	27.50±1.85	30.00±3.03	29.50±1.32	27.25±2.25	28.00±1.32	28.30±1.16
Lymphocytes (%)	66.00±2.31	69.25±1.03	67.75±2.63	67.50±1.19	69.75±1.65	68.30±1.21	67.80±1.11
Heterophil/Lymphocyte	0.40±0.00	0.40±0.03	0.44±0.06	0.44±0.03	0.39±0.04	0.41±0.02	0.42±0.02
Eosinophils (%)	1.50±0.29	1.50±0.87	0.25±0.25	1.50±0.50	0.75±0.48	1.00±0.40	1.20±0.29
Basophils (%)	0.50±0.29	0.25±0.25	0.50±0.29	0.50±0.29	0.75±0.25	0.50±0.17	0.50±0.17
Monocytes (%)	0.50±0.29	1.50± 0.29	1.50±0.65	1.00±0.41	1.50±0.65	1.20±0.36	1.20±0.25

<sup>a,b</sup> Means on the same row having different superscript are significantly ( $p < 0.05$ ) different.

PRV: 3 days pre-Newcastle disease vaccination administration of vitamins; PSV: 3 days post-Newcastle disease vaccination administration of vitamins

**Table 4:** Interactive effects of vitamins and administration periods on haematological parameters of broiler chickens at 14th day post-Lasota

Parameters	Control		A (0.45 mg/kg)		C (50mg/kg)		E (9.1mg/kg)		A+C+E (0.15+16.67+3.03 mg/kg)	
	PRV	PSV	PRV	PSV	PRV	PSV	PRV	PSV	PRV	PSV
Packed cell volume (%)	28.00±1.00	27.50±1.50	26.50±1.50	26.00±3.00	28.50±2.50	26.00±0.00	26.00±0.00	27.50±0.50	26.00±1.00	25.00±1.00
Haemoglobin (g/dl)	9.55±0.25ab	9.70±0.50a	8.30±0.30abc	8.35±0.65abc	9.60±0.70a	8.60±0.10abc	8.60±0.10abc	9.10±0.10abc	8.05±0.55bc	7.95±0.45c
Red blood cell (x10 <sup>18</sup> /μl)	2.50±0.10	2.35±0.35	1.95±0.35	2.05±0.55	2.70±0.50	2.25±0.15	2.25±0.15	2.50±0.00	1.95±0.15	1.75±0.05
Mean corpuscular volume (fl)	112.34±8.49	118.70±11.30	138.99±17.26	132.44±20.99	107.53±10.65	116.07±7.74	116.07±7.74	110.00±2.00	134.52±15.48	143.14±9.80
Mean corpuscular haemoglobin (pg)	38.30±2.53	41.89±4.11	43.70±6.30	42.97±8.36	36.32±4.13	38.42±3.01	38.42±3.01	36.40±0.40	41.75±6.03	45.54±3.87
Mean corpuscular haemoglobin concentration (g/dl)	34.12±0.33 <sup>ab</sup>	35.28±0.11 <sup>a</sup>	31.36±0.64 <sup>c</sup>	32.26±1.22 <sup>bc</sup>	33.73±0.50 <sup>ab</sup>	33.08±0.38 <sup>abc</sup>	33.08±0.38 <sup>abc</sup>	33.11±0.97 <sup>abc</sup>	30.93±0.93 <sup>c</sup>	31.78±0.53 <sup>bc</sup>
White blood cell (x10 <sup>15</sup> /μl)	9.70±0.70	13.80±2.40	9.85±0.95	11.20±0.20	12.85±0.05	13.35±1.25	13.35±1.25	10.40±1.80	11.00±0.80	9.95±1.05
Heterophils (%)	26.50±1.50	26.00±4.00	29.00±0.00	33.50±0.50	26.50±5.50	27.50±0.50	27.50±0.50	31.50±1.50	26.50±4.50	28.00±3.00
Lymphocytes (%)	66.00±4.00	70.50±1.50	68.00±1.00	64.00±1.00	71.50±3.50	69.50±0.50	69.50±0.50	65.50±0.50	71.50±2.50	68.00±2.00
Heterophil/Lymphocyte	0.40±0.00	0.37±0.06	0.43±0.01	0.52±0.02	0.38±0.10	0.40±0.00	0.40±0.00	0.48±0.03	0.37±0.08	0.41±0.06
Eosinophils (%)	1.50±0.50	1.50±1.50	1.50±1.50	0.00±0.00	0.50±0.50	2.00±1.00	2.00±1.00	1.00±0.00	0.00±0.00	1.50±0.50
Basophils (%)	0.50±0.50	0.50±0.50	0.00±0.00	0.50±0.50	0.50±0.50	0.50±0.50	0.50±0.50	0.50±0.50	0.50±0.50	1.00±0.00
Monocytes (%)	0.50±0.50	1.50±0.50	1.50±0.50	2.00±1.00	1.00±1.00	0.50±0.50	0.50±0.50	1.50±0.50	1.50±1.50	1.50±0.50

antioxidant potential and or the levels of the vitamins supplementation.

Vitamin C dietary supplementation significant influence on the erythrocytes indices (Mean corpuscular volume, Mean corpuscular haemoglobin) at 14th day post-Hitchner BI and Hb, content at 14th day post-Lasota, may be adduced to the ability of vitamin C to reduce the negative impact of environmentally imposed stress and thereby improving the physiological status of birds according to Chang et al. (1993). This is in agreement with the report of Oso et al. (2013) who concluded that supplementation of diet with vitamin C (2g/kg diet) improved the Packed cell volume, Haemoglobin, Red blood cell, and White blood cell of locally-adapted turkey. The significantly higher Haemoglobin and Mean corpuscular haemoglobin in birds fed with vitamin A supplemented diet three days pre-Lasota vaccine can be explained from the profound positive influence of vitamin A on organ development, cell proliferation and differentiation as reported by McDowell (2000). The Packed cell volume, Haemoglobin, Mean corpuscular volume, Mean corpuscular haemoglobin and Mean corpuscular haemoglobin concentration results in this study are within the normal reference range, Packed cell volume (22-35%), Haemoglobin (7-13 g/dl), Mean corpuscular haemoglobin (33-47 pg) and Mean corpuscular haemoglobin concentration (26-35%) for broiler chickens reported by Feldman et al. (2000). Mean corpuscular volume and Haemoglobin obtained are also consistent with those stated by Islam et al. (2004). The vitamins did not affect the white blood cell counts and its differentials at both 14th day post-Hitchner BI and post-Lasota, respectively. This is due to the positive influence of vitamins supplementation in ameliorating the negative effects of stressors on the birds. This is similar to the findings of Farooqi et al. (2005) who reported no significant difference in the white blood cells and the differential counts in birds fed diets supplemented with vitamins. Although the white blood cell differentials in this study were not significantly varied, the values obtained are within the normal range for healthy birds (Riddell, 2011).

## Conclusions

Dietary supplementation with vitamins C (50mg/kg) resulted to improved erythrocytes indices (Mean corpuscular volume and Mean corpuscular haemoglobin) in broiler chickens at 14th day post-Hitchner BI, while the use of vitamins A (9.1mg/kg) supplemented diet three days pre-Lasota vaccine resulted to better Haemoglobin and Mean corpuscular haemoglobin concentration in broiler chickens.

## Impact

The roles of vitamins in relation to administration periods in engendering sound health and physiological conditions in broiler chickens; thereby translating to increased production is presented in this study.

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## SEROPREVALENCE OF MAEDI -VISNA IN SHEEP IN SELECTED DISTRICTS OF AMHARA REGION , ETHIOPIA

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### Abstract

Maedi-Vsna(MV) causes a significant economical loss through morbidity, mortality and carcass weight loss in sheep worldwide. The present study was conducted to investigate the seroprevalence and risk factors of Maedi-visna in sheep in four selected districts of the Amharra region, Ethiopia. A total of 1536 sheep blood sera were randomly collected in the period from April, 2014 to December, 2015 and examined using indirect enzyme linked immune-sorbet assay (i-ELISA) to screen specific antibodies against *Maedi-Visna virus*. Of total samples tested, 61(4%) were positive for the presence of antibodies against *Maedi-Visna virus*. Considering the sensitivity and specificity of the test, the real seroprevalence calculated as 3.2%. The seroprevalence of Maedi visna was significantly different between sheep of different age groups ( $\chi^2 = 9.874$ ,  $P < 0.05$ ), different body condition scores ( $\chi^2 = 4.764$ ,  $p < 0.05$ ) and sheep with or without respiratory symptoms ( $\chi^2 = 279.56$ ,  $p < 0.05$ ). However, no significant difference was observed in the prevalence of Maedi-visna between sexes ( $\chi^2 = 0.002$ ,  $p > 0.05$ ) and different breeds of sheep ( $\chi^2 = 0.36$ ,  $p > 0.05$ ). Maedi Visna causes enormous economic losses. Thus, a comprehensive nationwide epidemiological investigation in areas were Awasi-Menze cross rams distributed for breeding purpose should be taken without delay to depict the real picture of the disease in the country .

**Key words:** Ethiopia, *Maedi-visna*, Risk factor, Sensitivity Seroprevalence, Specificity,

## SÉROPRÉVALENCE DE MAEDI -VISNA CHEZ LES OVINS DES DISTRICTS SÉLECTIONNÉS DANS LA RÉGION AMHARA EN ÉTHIOPIE

### Resume

La maladie causée par le virus Maedi-Vsna (MV) provoque des pertes économiques significatives en raison de la morbidité, de la mortalité et de la perte de poids de carcasse qu'elle engendre chez les ovins, partout dans le monde. La présente étude a été réalisée pour examiner la séroprévalence et les facteurs de risque de Maedi-visna chez les ovins dans quatre districts sélectionnés de la région d'Amharra en Éthiopie. Au total, 1536 sérums de sang d'ovins ont été prélevés de manière aléatoire entre avril 2014 et décembre 2015, et ont été examinés à l'aide d'un essai d'immuno-absorption enzymatique indirect (i-ELISA) pour détecter la présence d'anticorps spécifiques contre le virus *Maedi-Visna*. De l'ensemble des échantillons testés, 61 (4%) étaient positifs pour les anticorps contre le virus *Maedi-Visna*. Compte tenu de la sensibilité et de la spécificité du test, la séroprévalence réelle est de 3,2%. La séroprévalence de Maedi visna était significativement différente entre les moutons des différents groupes d'âge ( $\chi^2 = 9,874$ ,  $P < 0,05$ ), les différents scores d'état corporel ( $\chi^2 = 4,764$ ,  $p < 0,05$ ) et les moutons avec ou sans symptômes respiratoires ( $\chi^2 = 279,56$ ,  $p < 0,05$ ). Cependant, aucune différence significative n'a été observée au niveau de la prévalence de Maedi-visna entre les sexes ( $\chi^2 = 0,002$ ,  $p > 0,05$ ) et différentes races ovines ( $\chi^2 = 0,36$ ,  $p > 0,05$ ). Maedi-visna est à l'origine d'énormes pertes économiques. Ainsi, une enquête épidémiologique exhaustive à l'échelle nationale devrait être effectuée sans délai dans les zones où les béliers croisés Awasi-Menze distribués à des fins de reproduction, afin de décrire l'image réelle de la maladie dans le pays.

**Mots-clés :** Éthiopie, Maedi-visna, facteur de risque, sensibilité, séroprévalence, spécificité

## Introduction

Sheep rearing is an integral part of farming system in Ethiopia with the population of about 30 million (CSA, 2015). 34.2% of the country population of sheep is found in the Amhara region. This huge sheep population contributes about 30-35% of the agricultural gross domestic product (GDP) and foreign currency income through the exportation of live animals, meat and skin (CSA, 2015). However, due to low productivities of the indigenous breeds, the Ethiopian government had been introducing sheep breeds of Hampshire, Corriedale and Awassi from UK and Israel since the early 1970's to upgrade the genetic makeup of the local sheep breeds. Imported sheep were stocked and crossed with the local Menz and Horro breed sheep in Debre Berhan and Amed Guya sheep breeding and multiplication centers and cross-rams were distributed to different parts of the country (Getnet *et al.*, 2010). Despite the genetic improvement, the appearance of new respiratory disease complexes like Maedi-visna(MV) in the multiplication centers have forced the centers to stop breeding activities (Tibbo *et al.*, 2001)

*Maedi-Visna virus* (MVV) is a lentivirus belonging to the family Retroviridae. It was first reported in the Iceland and distributed worldwide (Radostits *et al.*, 2000; Straub, 2004), and causes non-suppurative encephalomyelitis (Visna) and chronic pneumonitis (Maedi) are the most frequently observed lesions in affected animals (Benavides *et al.*, 2009). Lifelong, persistent infections in sheep occur in the lungs, lymph nodes, spleen, joints, central nervous system, and mammary glands (Wu *et al.*, 2008). The main route of transmission is from an infected ewe to its lamb through colostrum and milk. Horizontal transmission via the respiratory route may also occur among sheep of any age, especially in high-density stocking situations (Prezioso *et al.*, 2004). As neither antiviral treatment nor vaccination is available, serological screening and culling of seropositive animals are the most implemented schemes to prevent the spread of MVV (Pépin

*et al.*, 1998).

Previous works which were conducted in and around the breeding and multiplication centers showed that MV became a paramount important respiratory disease of sheep (Getnet *et al.*, 2010; Gelagay *et al.*, 2001; Tibbo *et al.*, 2001). Even though, the existence of this disease is known since in the early 1990's, ranches have been distributed cross rams to users until recent past. South Wollo (Legambo and Woreilu districts) and North Shoa (Angolellatera and Basonaworena districts) administrative zones are amongst the area where these cross rams were distributed and the situation of the disease has not been well established yet in the areas. Therefore, this study was conducted to establish the sero-prevalence of MV in sheep and its associated risk factors in the study area.

## Material and Methods

### Study area

The study area was selected based on retrospective data showing the history of introduction of Awassi Menz cross rams. In four years period (2008-2011) about 5000 cross rams originated from sheep multiplication centers were distributed in different districts of S. Wollo and N. Shoa zones of Amhara region to upgrade the genetic potential of the local indigenous sheep breeds (BoARD, 2011). Of these districts, Legambo and Woreilu districts from S. wollo zone and Angolellatera and Basonaworena districts from N. Shoa zone were selected purposively for this study. Angolellatera and Basona-Worena districts are located at a distance of 110- 130 Kms North of Addis Ababa at a latitude between 9° 3' 26" to 9° 64' 92" N and 39° 14' 32" to 39° 27' 37" E longitude. Legambo and Woreilu the other study sites are situated about 500 Km North of Addis Ababa at a latitude between 10° 32' 86" to 10° 57' 81" N and 39° 14' 32" to 39° 26' 13" E longitude. The study districts are found in central highland of the country at an altitude of above 2770 m. The annual rain fall of the study area ranges from 950-1200mm. The mean annual minimum and maximum temperatures are 1.5 and 23.3°C, respectively and the area

experiences a bimodal rain fall patterns with a short rainy season which occurs from January to March and long rainy season which starts at the end of June and ends at early November.

#### Study animals

The study was carried out on 1536 local indigenous and Awasi-cross sheep breeds above six months of age and both sexes kept under extensive management system. Of these sheep, 768 were from two districts (Legambo and Woreilu) of S.wollo zone and 768 from two districts (Angolellatera and Basonawerana) of N. Shoa zone.

#### Study design and sample size determination

The study was carried out on four purposively selected districts of Amhara regional state and the samples were selected by simple random sampling methods. The desired sample size for this study was determined based on the expected prevalence 50% , the 5% desired absolute precision and 95% confidence interval(CI)) according to Thrusfield (2005).

$$n = \frac{1.96^2 * P_{ex}(1 - p_{ex})}{d^2}$$

Where

n = Required sample size

P<sub>ex</sub> = Expected prevalence

d = Desired absolute precision

1.962 = The value of z at 95% Confidence level  
Thus, the desired sample size for P<sub>ex</sub>= 0.5 is n =384. However, to increase accuracy and precision, 1536 sheep were included in this study .While collecting blood samples, data related to origin, sex, age, body condition and health status of each sampled animal were recorded properly. Age of the animals was determined from birth records (from owners) and based on detection formula as given by Abegaz and Awgichew (2009). Accordingly sheep with only milk teeth were classed as lambs ( less than 1 year old), and those with one to four pairs of permanent incisors were classed as young(>1year old), whereas sheep with a full set of teeth were classed as old.The

body condition scoring was classified according to Thompson and Meyer (1994) as lean (poor), medium and fatty (Good).

#### Sample collection

Blood samples were collected aseptically from jugulars vein using disposable needles and non-heparinized vacutained tubes and then brought to Kombolcha regional veterinary laboratory in an icebox, where blood samples were kept overnight to clot at slant position at room temperature. Then the separated serum was carefully collected in cryovial without mixing with the collected blood. The serum was labeled and stored at -200C until further processing took place

#### Sample processing

A commercially available i-ELISA diagnostic kit (IDvet, innovative diagnostics) was used in accordance with the manufacturer's instructions manual (VISNAB ver 1213 GB) to determine the presence of specific antibodies against MVV. The sensitivity and specificity values for the test were 91.7% and 98.9% respectively. The test was performed at National Animal Health Disease Investigation Center (NAHDIC), Sebeta, Ethiopia.

#### Data Management and Analysis

All collected data were entered and organized into Microsoft-Excel spreadsheet program and analyzed using SPSS-20 software version. Descriptive statistics such as percentage was used to approximate the seroprevalence for MVV antibodies in the study area. Considering the sensitivity and specificity of the test the real animal level seroprevalence was also calculated by the following formula (Rogan and Gladen, 1978).

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

Where: AP = Apparent prevalence, Se =Sensitivity of the test series, Sp = specificity of the test series.

The relationship of associated risk factors with positive serological test result was analyzed by logistic regression. A test value at  $p < 0.05$  was taken as statistical significant.

## Result

### Maedi Visna Seroprevalence

In the present study a total of 1536 sheep serum samples (748 from local indigenous and 788 from Awassi- cross sheep breeds) were collected from four districts of North Shoa and South Wollo zones to screen specific antibodies for MVV using i-ELISA serological test. Of total samples tested, 61 (4%) were positive for the presence of antibodies against MVV. Considering sensitivity and specificity of the test, the estimated overall adjusted true animal level sero-prevalence calculated as 3.2%. The highest and the lowest

sero-positivity rate were in Legambo (6.3%) and Angollelatara and Woreilu districts (2.3%), respectively (Table 1).

### Risk factors

The univariable logistic analysis of putative risk factors indicated a significance difference in sero-positivity between sheep of different age groups ( $\chi^2 = 9.874$ ,  $p < 0.05$ , OR = 2.392), different body condition scores ( $\chi^2 = 4.764$ ,  $p < 0.05$ , OR = 1.815) and sheep with or without respiratory symptoms ( $\chi^2 = 279.56$ ,  $p < 0.05$ , OR = 34.23). However, no significant difference was observed in the prevalence of MV infection between the male sheep (4.0%) and female sheep (3.95%) ( $\chi^2 = 0.002$ ,  $p > 0.05$ ). Similarly, there was no significant difference in sero-prevalence between different sheep breeds ( $\chi^2 = 0.36$ ,  $p > 0.05$ ) (Table 2).

**Table 1:** Seropositivity to MVV antibodies in sheep detected by i-ELISA from study districts

Districts	No samples	No positive	Apparent Prevalence rate (%)	True prevalence (%)
Legambo	384	26	6.8	6.3
Basonaworena	384	17	4.4	3.6
Angollelatara	384	9	2.4	2.3
Woreilu	384	9	2.4	2.3
<b>Total</b>	<b>1536</b>	<b>61</b>	<b>4.0</b>	<b>3.2</b>

## Discussion

Maedi vsna causes a significant economical loss through morbidity, mortality and carcass weight loss in sheep worldwide. The result of the present study conducted in four districts of Amhara region, Ethiopia disclosed an overall adjusted true animal level sero-prevalence of 3.2% Maedi-visna infection in sheep. The 3.2% prevalence of MV in this study is in line with the reports of Getnet et al., (2002) 6% in north Omo, Ethiopia, Mahin et al., (1984) 2.7% in Morocco, Sihvonen et al., (1999) 1.6% in Finland, Shuaib et al., (2010) 2.41% in Manitoba, Canada and Aslantas et al., (2002) 1-5-2.6% in Hatay region, turkey. However, the seroprevalence result of the present study is much lower than many

of the previous reports in Ethiopia, viz. 74% in central Ethiopia (Woldemeskel et al., 2002), 62.5% in central cool highland (Garedew et al., 2010), 70.4% in Sheno agricultural research center (Seyoum et al., 2011), 15.6% in eastern Amhara region (Tsegaw and Ademe, 2012) and 88% and 20% in Debre-Brhan sheep breeding center and Arsi, respectively (Getnet, et al., 2010). The findings in this study were also much lower than in other countries of the world. For instance, a prevalence of 15.3% was reported in Turkish sheep (Prezioso, et al., 2010), 15.6% in culled ewes in Alberta, Canada (Fournier et al., 2006), 29.6% in Khorasan-e-Razawi province, Iran (Norouzi et al., 2015), 28.8% in Germany (Hüttner et al., 2010), 50% in Palestine (Hananeh and Barhoom, 2009), 19% in Canada (Simard and Morley, 1991), 19.4% in

**Table 2:** Sero-positivity to MV infection in sheep based d on various risk factors

Risk factors	No sampled	No positive	Prevalence (%)	$\chi^2$	p-value	OR	95.0% CI
Sex							
Male	349	14	4.0				
Female	1187	47	3.95	0.002	0.965	0.987	0.537-1.84
Breed							
Local	788	29	3.7				
Awassi-cross	748	32	4.3	0.36	0.549	1.855	0.512-1.428
Age							
Young	756	18	2.4				
Old	780	43	5.5	9.874	0.002	2.392	1.367-4.186
Body condition							
Good	1184	40	3.4				
Poor	352	21	6.0	4.764	0.029	1.815	1.055-3.120
Respiratory symptoms							
Without R. symptom	1379	16	1.2				
With R. symptom	157	45	28.7	279.56	0.000	34.23	18.744-62.500

Kirikkale district, Turkey (Azkur *et al.*, 2011) and 18% in Wyoming sheep, USA (Gerstner *et al.*, 2015). Such inconsistency in the prevalence rates of MV may be due to the variation in the diagnostic tests, sampling method used, the prevalence variability within the population studied, the characteristics of the animals forming the population, susceptibility of different breeds to the disease, management practices and measures taken to control the disease.

This survey showed a variation in sero-prevalence of MV between different study districts (2.4% to 6.8%). Similar results were obtained in different parts of Quebec (14.5% to 69 %) (Shuaib *et al.*, 2010), in turkey (3.8% to 41.2%) (Alkan and Tan, 1998), in Iran (6.7% to 72.2%) (Norouzi *et al.*, 2015) and indifferent parts of Ethiopia (0.6% to 88%) (Getnet *et al.*, 2010). This geographic difference in distribution of positive cases could be explained by the introduction of carrier animals from an infected area to disease free zones, the management practices and the bio-security followed by farm owners.

There was no statistically significant difference in seroprevalence between male and female sheep ( $\chi^2 = 0.002$ ,  $P > 0.05$ ), which is in agreement with findings of Woldemeskel *et al.*, (2002) and Seyoum *et al.*, (2011). The breed related seroprevalence of MV infection in present study showed no statistical significant difference between breeds ( $\chi^2 = 0.36$ ;  $P > 0.05$ ). The possible explanation for this similarity could be due to the fact that animals rearing condition in the study areas was identical (extensive) and different breeds were herded together without separation and sheep of different breeds were in direct contact with each other. In contrast our finding, susceptibility difference were reported between Menz and Awassi sheep breeds (Seyoum *et al.*, 2011; Tsegaw and Ademe., 2012). This breed susceptibility difference could be related to the influence of traits of particular family lines, the strain of the virus and the result of one or more recessive genes (Simard and Morley 1991).

The result obtained in the present study disclosed that older sheep were about 2.4 (95% CI= 1.367-4.186). times more likely to be infected as compared to younger sheep. In this regard, the finding of this study is consistent with the results reported elsewhere. viz, in Canada (Arsenault et.al., 2003 ; Simard and Morley, 1991), in Ethiopia ( Ayelet et.al.,2001), in Turkey ( Preziuso et al.,2010) and in Iran (Norouzi et al.,2015). This age sero-prevalence discrepancy can probably be explained by the longer exposure to horizontal transmission and development of detectable levels of MV antibodies can vary from months to years (Radostits et.al.,2000). Thus, the older the animals, the greater the potential for a greater proportion of sheep to be become infected with MV.

In the present study, an attempt was carried out to know whether body condition influence or not on prevalence of MV infection in sheep; and it was found that poor body condition animals were about 2.0 (95% CI= 1.055-3.120) times more likely to be infected as compared to good body condition animals. Our finding is in accordance with the finding of Pritchard and Dawson (2000) who reported sever emaciation in sheep infected with MV. This is supported by the fact that MVV targets the cells of the immune system leading to concomitant infectious diseases and ultimately weight losses.

The present study disclosed that animals with respiratory symptoms were about 34 (95% CI = 18.744-62.500) times more likely to be infected by MV than animals without clinical symptoms. Similarly, higher MV prevalence were reported in sheep with respiratory symptoms in Canada, (Arsenault et. al., 2003; Fournier et al., 2006), in Ethiopia (Getnet et al., 2010). The occurrence of such symptoms in MV affected animals could be explained by histopathological changes including chronic interstitial inflammation with diffuse thickening of the inter alveolar septa along with infiltration of large mononuclear cells leading to the total obliteration of the alveoli. Hyperplasia of the epithelium in small bronchioles is often observed (Hananeh and

Barhoom, 2009)

## Conclusion

In conclusion, our findings showed that MV is relatively less prevalent in North Shoa and South Wollo of Amhara Regional state; however the economic losses could be enormous. Therefore, we strongly recommended for detail nationwide epidemiological investigation in areas were Awasi-Menze cross rams distributed for breeding purpose and further strict serological screening of these breeds for MV is highly recommended before their distribution.

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## CARCASS CHARACTERISTICS AND MEAT QUALITY OF RABBIT LITTERS FROM RABBIT DOES RESTRICTED DURING PREGNANCY

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### Abstract

The effect of restricted feeding and realimentation during pregnancy was studied to know the carryover effect on carcass characteristics and meat quality of rabbit litters. Young does fed ad libitum diets often show parturition problems (Dystokia and abnormal presentation) with the subsequent reduction of number of kits, linked to excessive fatness; thus this study aims to know whether feed restriction during pregnancy have effect on carcass characteristics and meat quality of rabbit litters. The carcass and meat quality of rabbit litters whose does were restricted during pregnancy were examined for a period of sixteen weeks. A total of one hundred and eight litters of mixed breeds and sexes from rabbit does that were restricted during pregnancy were used for this study. These rabbit does were exposed to three levels of quantitative feed restriction (0, 15 and 30%) at three different periods of gestation (15-19, 20-24 and 25-29 days). At weaning three rabbits were selected from each rabbit doe which was subdivided into 4 replicates of 3 rabbits each; thus making 9 treatments groups of 12 rabbits each. All rabbit litters from each treatments were fed ad libitum throughout the experimental period. Carcass characteristics and meat quality were evaluated at the end of sixteen weeks of age. The results obtained on main effect for carcass characteristics showed that significant ( $p < 0.05$ ) differences were obtained on neck, chest, loin, back, lungs and liver for the levels and periods of feed restriction while other parameters measured were not significantly influenced ( $p > 0.05$ ). Results obtained on interaction shows that significant ( $p < 0.05$ ) differences were obtained on carcass weight, neck, hindlimbs, chest, loin, back, lungs, heart, liver and spleen while other parameters measured were not significantly influenced ( $p > 0.05$ ). Highest carcass (1294.16g) weight was obtained from growing rabbits from rabbit does on 30% restriction between 25-29 days of gestation. The result obtained on main effect and interaction on meat quality shows that all parameters measured were not significantly influenced by the treatment means ( $p > 0.05$ ). This result depicts that feeding levels during pregnancy did not have any effect on meat quality. In conclusion feed restriction during pregnancy resulted into higher carcass weight, dressing percentage at the end of the post weaning experiment. Feed restriction during pregnancy resulted in similar mean values for moisture, total cholesterol, pH and crude protein; thus feed restriction can be applied on pregnant does at 15% or 30% level between 20-24 days or 25-29 days of gestation as this level and period gave better carcass yield.

**Keywords:** Growing Rabbits, restriction, carcass characteristics, and meat quality.

## CARACTERISTIQUES DES CARCASSES ET QUALITE DE LA VIANDE DES LAPERAUX ISSUS DE LAPINES SOUMISES A UNE RESTRICTION ALIMENTAIRE PENDANT LA GESTATION

### Résumé

L'effet de la restriction alimentaire et de la réalimentation pendant la gestation a été étudié pour déterminer ses répercussions sur les caractéristiques des carcasses et la qualité de la viande des lapereaux. Les jeunes lapines soumises à une alimentation ad libitum montrent souvent des problèmes de

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parturition (dystocie et présentation anormale) avec une réduction subséquente du nombre de lapereaux liée résultant d'un excès de graisses. Cette étude cherche donc à savoir si la restriction alimentaire pendant la gestation a un effet sur la carcasse et la qualité de la viande des lapereaux. La carcasse et la qualité de la viande des lapereaux dont les mères ont été soumises à une restriction alimentaire pendant la gestation ont été examinées pendant une période de seize semaines. Un total de cent huit lapereaux de races mixtes et des deux sexes, dont les mères avaient été soumises à une restriction alimentaire pendant la gestation, a été utilisé pour cette étude. Ces lapines avaient été exposées à trois niveaux de restriction alimentaire quantitative (0, 15 et 30%) à trois périodes de gestation différentes (15-19, 20-24 et 25-29 jours). Au sevrage, trois lapereaux ont été sélectionnés pour chaque lapine, et ont été subdivisés en 4 répétitions de 3 lapereaux chacune; constituant ainsi 9 groupes de traitements de 12 lapereaux chacun. Tous les lapereaux dans chaque traitement ont été nourris ad libitum tout au long de la période expérimentale. Les caractéristiques des carcasses et la qualité de la viande ont été évaluées à la fin des seize semaines d'âge. Les résultats obtenus à propos de l'effet principal sur les caractéristiques des carcasses ont montré des différences significatives ( $p < 0,05$ ) pour le cou, la poitrine, la longe, le dos, les poumons et le foie pour les niveaux et les périodes de restriction alimentaire, tandis que les autres paramètres mesurés n'étaient pas influencés de façon significative ( $p > 0,05$ ). Les résultats obtenus lors de l'interaction montrent que des différences significatives ( $p < 0,05$ ) ont été observées sur le poids de la carcasse, le cou, les membres postérieurs, la poitrine, la longe, le dos, les poumons, le cœur, le foie et la rate, tandis que les autres paramètres mesurés n'étaient pas influencés de manière significative ( $p > 0,05$ ). Le poids de carcasse le plus élevé (1294,16 g) a été obtenu chez les lapereaux en croissance issus de lapines soumises à 30% de restriction entre 25-29 jours de gestation. Le résultat obtenu sur l'effet principal et l'interaction sur la qualité de la viande montre que tous les paramètres mesurés n'ont pas été influencés de façon significative par les moyens de traitement ( $p > 0,05$ ). Ce résultat montre que les niveaux d'alimentation pendant la gestation n'ont eu aucun effet sur la qualité de la viande. En conclusion, la restriction alimentaire pendant la gestation a entraîné une augmentation du poids de la carcasse, du rendement en carcasse à la fin de l'expérience post-sevrage. La restriction alimentaire pendant la gestation a donné des valeurs moyennes semblables pour l'humidité, le cholestérol total, le pH et la protéine brute. Par conséquent, la restriction alimentaire peut être appliquée aux lapines gestantes au niveau de 15% ou 30% entre 20-24 jours ou 25-29 jours de gestation, car ce niveau et la période ont donné un meilleur rendement en carcasse.

**Mots-clés :** lapins en croissance, restriction, caractéristiques des carcasses, qualité de la viande

## Introduction

Rabbit production has gained considerable interest recently in Nigeria because of the exorbitant prices of the conventional sources of meat, such as cattle (beef), goats (chevon), sheep (mutton), pig (pork) and poultry. Rabbits are renowned for their fecundity and prolificacy (Biobaku and Dosunmu, 2003); ability to utilize forages (Aduku and Olukosi, 1990). Rabbit meat is low in fat and cholesterol (Biobaku and Oguntona, 1997) thus making the flesh a desirable one for diabetics, hypertensive and middle aged people. Rabbit meat is an important source of protein for human because of its high quality and low fat. Meat from rabbit is highly digestible, tasty, low-caloried and often recommended by nutritionists over other meat types. It has been

reported that rabbits, especially the newly weaned ones should not be maintained on sole forage without a little supplement of a balance concentrate. This is to guarantee maximum productivity (Ojebiyi et al., 2006). However, inadequate and high cost of feed ingredients brought about mainly by the stiff competition between man and monogastric animals such as rabbits and poultry for grains is the major constraint to rabbit production (Agunbiade et al., 2002). In order to address this problem, lhekumwumere et al. (2004) stressed the need to find alternative ways, which are cheap, adequate and readily available for feeding livestock. Also, feed restriction could be exploited in the feeding regimen of rabbits, especially in periods of inadequate supply of concentrates and forages (Yakubu et al., 2007). There has been an increased interest in studying feed restriction

in broiler rabbits as a means of reducing the cost of production. Early feed restriction also helps to address problems associated with early-life fast growth rate such as increased body fat deposition, high incidence of metabolic disorders and high mortality (Urdaneta-Rincon and Leeson, 2002). However, limiting the feed intake is widespread in animal breeding such as for adjusting the ration to the nutrient requirements or to manage the fattening and the meat quality (Gidenne et al, 2009). However, feed restriction became systematic in much country as a preventive method against post-weaning digestive disorders (Gidenne et al, 2003; Boisot et al, 2004 and Bergaoui et al, 2008). Boisot et al. (2003) demonstrated the interest of a preventive restricted feeding to reduce the negative effect of this disorder on the growth performance of rabbits. The effect of feed restriction on meat quality depends on implementation, i.e. on the intensity of feed restriction, its duration, and age when it is applied. The limited feed intake decreases growth in the period of restriction. Gidenne et al. (2009) stated that a linear decreasing of growth is about 0.5 g/day per each percentage of feed reduction. Following the restriction, rabbits are fed ad libitum (ADL) and it can exhibit in higher daily weight gain typical for compensatory growth (Tumová et al., 2002, 2003). Compensatory growth is defined as a physiological process whereby an organism accelerates its growth after a period of feed restriction. The range of compensatory growth may be quantified by the "compensatory index". Compensatory index (I) is the ratio of the difference between weight variation at the end of restricted growth (A) and compensatory growth (B) periods, respectively, relative to the variation at the end of restricted growth:  $I = (A - B) / A$  (Hornick et al., 2000; Andersen et al., 2005). Carcass characteristics are important factors to consider when evaluating alternative feeding programs. Ledin et al. (1984a) concluded that carcass and dissection characteristics were not influenced by restriction. According to Perrier and Ouhayoun (1996) rabbits restricted from 56 days of age had lighter carcasses but carcass yield was the same as in the ad libitum

fed rabbits. Rabbits restricted till 56 days had better carcass yield. In (Tumová et al., 2003) experiment on time restriction they reported that time of restriction did not affect carcass weight and dressing percentage. Quantitatively restricted rabbits with a restriction period of 3 weeks had significantly lower ( $P < 0.05$ ) Carcass weight than the groups restricted one or two weeks, but dressing percentage was not significantly ( $P > 0.05$ ) influenced by the feeding regime. As organs perform different metabolic function and grow at a different rate, undernutrition may affect the growth and development of some organs and tissues. Thus this study aims to investigate the effect of quantitative feed restriction during pregnancy on carcass yield and meat quality of post-weaned rabbits.

## Materials and Methods

### Experimental Site

The experiment was carried out at the Rabbitary Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State. The site is located in the rain forest vegetation zone of South-Western Nigeria on latitude 7° 13' 49.46" N, longitude 3° 26' 11.98E and altitude 76m above the sea level. The climate is humid with a mean annual rainfall of 1037mm and mean temperature and humidity of 34.7°C and 83%, respectively (Google Earth, 2014).

### Experimental Animals and Management

All rabbit litters present with their does were raised during the pre-weaning period that lasted for 6 weeks. One hundred and eight kits were used for the post-weaning experiment that lasted for 10 weeks. Three rabbits were selected from each rabbit doe which was subdivided into 4 replicates of 3 rabbits each; thus making 9 treatments groups of 12 rabbits each. Each group of this rabbits were fed ad libitum each day.

### Experimental Design

Each pregnant doe was exposed to three levels of restricted concentrate feeding

(0, 15 and 30%) at three periods of restriction (15-19, 20-24, 25-29 days) in a 3 x 3 factorial arrangement in a one way analysis of variance. Composition of concentrate diet fed during pregnancy and after pregnancy is shown in table I. After kindling of does all kits were fed ad libitum before weaning and after weaning. The composition of concentrate diet fed to growing rabbit after weaning is shown in table I under B. Quantitative feed restriction fed to pregnant does was in the following order :

0% Restriction (control) was fed (100 g/ rabbit/day). (Ad libitum fed)

15% Restriction was fed (85 g/rabbit/day).

30% Restriction was fed (70 g/rabbit/day).

**Table I:** Composition of concentrate diet (% as fed)

Ingredients	A	B
	%	%
Maize	47.50	48.00
Fish meal	2.00	2.00
Soybean meal	3.00	3.00
Wheat offal	23.00	10.00
Groundnut cake	12.00	14.00
Rice husk	7.00	20.00
Bone meal	3.00	1.50
Oyster shell	2.00	1.00
Salt	0.25	0.25
Vitamin and Mineral premix	0.25	0.25
	100	100.00
<b>Determined Analysis</b>		
ME (Kcal/kg)	2578.8	2591.80
Ash (%)	2.74	2.74
Crude fibre %	10.65	15.50
Crude protein	16.20	15.80
Nitrogen free extract	42.50	40.50

Note: premix: Vit A 8000 iu, Vit D3 2000 iu, Vit E 5 iu, Vit K 2 mg, Riboflavin 4.20 mg, Vit B12 0.01 mg, Pantothenic acid 5 mg, Nicotinic acid 20 mg, Folic acid 5 mg, Choline 300 g, Mn 56 mg, Fe 20 mg, Cu 10 mg, Zn 50 mg.

A- Composition of concentrate diet fed to rabbit does during pregnancy  
 B- Composition of concentrate diet fed to post-weaned rabbits

### Carcass yield

At the end of the 16th week of the experiment, all the one hundred and eight (108) rabbits were slaughtered and used to determine the carcass yield and meat quality. Feed was withdrawn for 12 hours from the rabbits so as to empty their gastro intestinal tract (GIT) and to reduce the variability in body weight due to intestinal content. Prior to slaughtering, the rabbits were weighed, stunned, bled and skinned as described by Omojola and Adesehinwa (2006), and then eviscerated, washed and weighed according to (Okubanjo, 1997). The carcass weight and dressing percentage were determined and recorded.

$$\text{Dressing percentage} = \frac{\text{Carcass weight}}{\text{Live weight}} \times 100$$

The carcasses were chilled at 40 C for 24 hours and cut into retail parts (fore limb, hind limb, chest, neck, loin, back, head and tail (sacral region to the tip of the tail) according to Aduku and Olukosi (1990) and were weighed with a electronic sensitive scale. The weights of the liver, kidney, heart and lung were also taken and expressed as percentage of live weight.

### Proximate composition and pH of meat

Proximate composition of rabbit meat was determined following the procedures of A.O.A.C (2000). Fresh samples of meat were taken individually from the thigh muscle of each rabbit for proximate composition. Moisture was determined by drying 2g of meat in an oven at 100–1050C until a constant weight was obtained. Crude protein was determined by using kjedahl method which comprised, digestion distillation, and titration of the distillate. Crude protein value was obtained by converting nitrogen (N%) content obtained with a constant (6.25), thus crude protein was obtained as (6.25 x N%). The pH of meat was measured with pH meter model HI8424 microcomputer, Havana Instruments Romania (Marchiori and deFelicio 2003). Total cholesterol value of meat was detected after extraction of lipids by the method of Folch (1957).

**Table 2:** Main effect of level and period of feed restriction during gestation on carcass characteristics of post-weaned growing rabbits

Parameters	Level of feed restriction of does			Period of feed restriction of does			SEM
	0%	15%	30%	15-19 days	20-24 days	25-29 days	
Fasted live weight (g/rabbit)	1354.16	1269.44	1283.33	1269.44	1300.97	1336.52	30.22
Carcass weight (g/rabbit)	1251.27	1179.37	1197.15	1185.75	1197.11	1244.94	30.35
Dressing percentage (%)	57.73	59.05	59.13	58.72	58.09	59.11	0.87
<b>Cut up parts (%)</b>							
Head	8.92	8.66	8.86	9.03	8.60	8.79	0.22
Neck	1.94b	2.16a	2.18a	2.23a	2.12a	1.93b	0.08
Forelimb	10.72	10.42	10.79	10.90	10.27	10.69	0.23
Hindlimb	18.10	17.46	17.84	18.08	17.47	17.86	0.30
Chest	6.29c	7.99b	8.72a	7.96a	7.36b	7.67ab	0.20
Loin	7.69a	7.44b	8.55a	8.13	7.80	7.75	0.19
Back	14.94a	12.56b	12.08b	13.28	13.01	13.29	0.32
Tail	0.58a	0.44b	0.43b	0.50a	0.45b	0.49a	0.01
<b>Organs</b>							
Lungs	0.78b	0.88a	0.91a	0.87	0.90	0.80	0.04
Heart	0.22	0.24	0.25	0.23	0.25	0.23	0.09
Kidney	0.79	0.77	0.80	0.82	0.75	0.79	0.03
Liver	1.90b	2.43a	2.44a	2.34a	2.41a	2.01b	0.10
Spleen	0.05	0.07	0.07	0.07	0.06	0.06	0.04

<sup>abc</sup> Means in the same row with different superscripts differ significantly ( $p < 0.05$ )

SEM: standard error of mean

**Table 3:** Interaction between level and period of feed restriction during breeding on carcass characteristics of post-weaned growing rabbits

Level of feed restriction of does	0%			15%			30%			SEM
	Period of feed restriction of does			Period of feed restriction of does			Period of feed restriction of does			
	Days	Days	Days	Days	Days	Days	Days	Days	Days	
<b>Parameters</b>										
Fasted live weight (g/rabbit)	1362.50	1348.75	1351.25	1200.00	1329.16	1279.16	1245.83	1225.00	1379.16	45.77
Carcass weight (g/rabbit)	1258.50 <sup>ab</sup>	1249.66 <sup>ab</sup>	1245.66 <sup>ab</sup>	1127.91 <sup>b</sup>	1215.20 <sup>ab</sup>	1195.00 <sup>ab</sup>	1170.83 <sup>ab</sup>	1126.45 <sup>b</sup>	1294.16 <sup>a</sup>	50.24
Dressing percentage (%)	57.93	58.56	56.69	58.87	58.06	60.20	59.35	57.63	60.42	1.37
<b>Cut up parts (%)</b>										
Head	8.88	8.84	9.03	8.99	8.40	8.58	9.22	8.58	8.77	0.42
Neck	1.91 <sup>b</sup>	1.98 <sup>b</sup>	1.93 <sup>b</sup>	2.41 <sup>a</sup>	2.14 <sup>ab</sup>	1.92 <sup>b</sup>	2.37 <sup>a</sup>	2.24 <sup>ab</sup>	1.94 <sup>b</sup>	0.10
Forelimb	11.39	10.34	10.45	10.54	10.31	10.40	10.98	10.15	11.23	0.40
Hindlimb	17.84 <sup>ab</sup>	18.55 <sup>a</sup>	17.98 <sup>b</sup>	18.53 <sup>a</sup>	16.44 <sup>b</sup>	17.42 <sup>ab</sup>	17.86 <sup>ab</sup>	17.40 <sup>ab</sup>	18.26 <sup>a</sup>	0.51
Chest	6.27 <sup>d</sup>	6.28 <sup>d</sup>	6.32 <sup>d</sup>	8.21 <sup>bc</sup>	8.00 <sup>bc</sup>	7.75 <sup>c</sup>	9.41 <sup>a</sup>	7.80 <sup>c</sup>	8.94 <sup>ab</sup>	0.32
Loin	7.71 <sup>b</sup>	7.62 <sup>b</sup>	7.72 <sup>b</sup>	7.73 <sup>b</sup>	8.11 <sup>ab</sup>	6.47 <sup>c</sup>	8.94 <sup>a</sup>	7.66 <sup>b</sup>	9.04 <sup>a</sup>	0.34
Back	15.13 <sup>a</sup>	14.76 <sup>a</sup>	14.93 <sup>a</sup>	12.79 <sup>b</sup>	12.19 <sup>b</sup>	12.71 <sup>b</sup>	11.90 <sup>b</sup>	12.08 <sup>b</sup>	12.25 <sup>b</sup>	0.52
Tail	0.58 <sup>a</sup>	0.57 <sup>a</sup>	0.61 <sup>a</sup>	0.46 <sup>b</sup>	0.40 <sup>bc</sup>	0.43 <sup>bc</sup>	0.46 <sup>b</sup>	0.39 <sup>c</sup>	0.43 <sup>bc</sup>	0.02
<b>Organs</b>										
Lungs	0.76 <sup>bc</sup>	0.77 <sup>bc</sup>	0.79 <sup>abc</sup>	0.94 <sup>ab</sup>	0.95 <sup>ab</sup>	0.74 <sup>c</sup>	0.89 <sup>abc</sup>	0.97 <sup>a</sup>	0.87 <sup>abc</sup>	0.06
Heart	0.23 <sup>abc</sup>	0.23 <sup>abc</sup>	0.19 <sup>c</sup>	0.24 <sup>abc</sup>	0.25 <sup>ab</sup>	0.24 <sup>abc</sup>	0.21 <sup>bc</sup>	0.26 <sup>a</sup>	0.27 <sup>a</sup>	0.02
Kidney	0.82	0.78	0.76	0.79	0.77	0.76	0.83	0.71	0.86	0.04
Liver	2.05 <sup>cd</sup>	1.93 <sup>cd</sup>	1.72 <sup>d</sup>	2.49 <sup>ab</sup>	2.76 <sup>a</sup>	2.04 <sup>cd</sup>	2.49 <sup>ab</sup>	2.54 <sup>ab</sup>	2.28 <sup>bc</sup>	0.14
Spleen	0.05 <sup>c</sup>	0.05 <sup>c</sup>	0.05 <sup>c</sup>	0.07 <sup>b</sup>	0.08 <sup>a</sup>	0.06 <sup>bc</sup>	0.09 <sup>a</sup>	0.06 <sup>bc</sup>	0.06 <sup>bc</sup>	0.08

<sup>a,b,c</sup>Means in the same row with different superscripts differ significantly ( $p < 0.05$ )  
SEM: Standard error of mean

### Statistical Analysis

Data collected were arranged in a 3×3 factorial experimental layout and then subjected to one way analysis of variance (SAS, 1999). Significantly ( $p<0.05$ ) different means were separated using Duncan's Multiple Range Test of the same statistical package.

### Results

Table 2 shows the main effect of level of feed restriction and period of feed restriction during pregnancy on carcass characteristics. The level of feed restriction of does had no significant ( $p>0.05$ ) effect on carcass weight, dressing percentage, fore and hind limbs weights, head, spleen, heart and kidney. However, the level of feed restriction of does have significant ( $p<0.05$ ) effect on liver, lungs, chest, neck, loin, tail and back weights. The loin and back were also significantly ( $p<0.05$ ) affected by level of feed restriction; growing rabbits from does on 30% level of feed restriction recorded the highest value of 8.55% for loin weight compared to 7.44% noted for growing rabbits from does on 15% level of feed restriction. The back weight of the growing rabbits also differs across the dietary treatments with growing rabbits from does on 0% level of feed restriction recording a higher value (14.94%) compared to the value of 12.08% obtained for growing rabbits from does on 30% level of feed restriction.

The period of restriction of does had no significant ( $p>0.05$ ) effect on carcass weight, dressing percentage, heart, kidney, spleen, lungs, forelimbs, hindlimbs, head, loin and back. However, significant ( $p<0.05$ ) differences were obtained on the liver, chest, neck and tail. Growing rabbits from does restricted between 20-24 days had higher values (2.41%) for liver compared to growing rabbits from does restricted at 25-29 days of gestation period that recorded the least (2.01%). The chest weight of the growing rabbits differs across the period of feed restriction. Growing rabbits from does restricted between 15-19 days had higher values 7.96% for chest compared to growing rabbits from does restricted between 20-24 days that have the least value 7.36%.

Table 3 shows the effect of interaction between level of feed restriction and period of feed restriction on carcass characteristics. Significant ( $p<0.05$ ) differences were obtained on carcass weight, neck, hindlimb, chest, loin, back, tail, lungs, heart, liver and spleen. However, there were no significant ( $p>0.05$ ) differences on dressing percentage, head, forelimbs and the kidney. Carcass weight differed ( $p<0.05$ ) across the treatments, with range values of 1,294.16g to 1,127.91g for growing rabbits from does on 30% level of feed restriction at 25-29 days of gestation having the highest mean value 1294.16 g. Growing rabbit from does on 30% level of feed restriction between 25-29 days had highest mean value 60.42% for dressing percentage compared to 56.69 % noted for the offspring of does on 0% level of feed restriction at 25-29 days of gestation period. The heart (%) of growing rabbit from does on 30% level of feed restriction between 25-29 days had higher mean value 0.27% compared to rabbits from does on 0% level of feed restriction between 25-29 days that recorded the least value 0.19%. Growing rabbits from does on 0% level of feed restriction between 20-24 days recorded the highest mean value 18.55% live weight for hindlimbs compared to 16.44% live weight obtained for growing rabbit from does on 15% level of restriction 20-24 days. The chest of the growing rabbit also differs across the dietary treatment with rabbits from does on 30% level of feed restriction, between 15-19 days having the highest mean value 9.41% while growing rabbits from does on 0% level of feed restriction, between 15-19 days recorded the lowest value 6.28%. Higher mean value 9.04% for loin were recorded in growing rabbits from does on 30% level of feed restriction between 25-29 days compared to 6.47% obtained for growing rabbits from does on 15% level of feed restriction between 25-29 days that recorded the least.

Table 4 shows the main effect of level of feed restriction and period of feed restriction on meat quality of post weaned rabbits. The levels and periods of feed restriction during pregnancy had no significant ( $p>0.05$ ) effect on moisture, total cholesterol, pH and crude

**Table 4:** Main effect of levels and periods of feed restriction during gestation on meat quality of post-weaned growing rabbits

Parameters	Level of feed restriction of does			Period of feed restriction of does				
	0%	15%	30%	SEM	15-19 days	20-24 days	25-29 days	SEM
Moisture (%)	65.98	65.63	66.20	0.57	65.95	66.21	65.65	0.75
Total cholesterol (mg/dl)	54.60	54.68	54.54	9.05	53.90	55.00	54.91	0.65
pH	5.28	5.75	5.55	0.55	5.45	5.41	5.71	0.45
Crude protein (%)	20.41	20.79	20.69	1.65	20.71	20.97	20.21	1.55

**Table 5:** Main effect of levels and periods of feed restriction during gestation on meat quality of post-weaned growing rabbits

Level of feed restriction of does	0%			15%			30%			
	15-19 Days	20-24 Days	25-25 Days	15-19 Days	20-24 Days	25-29 Days	15-19 Days	20-24 Days	25-29 Days	SEM
Moisture (%)	65.25	67.25	65.45	66.75	65.15	65.00	65.85	66.25	66.52	0.55
Total cholesterol (mg/dl)	55.00	54.55	54.25	53.45	55.15	55.45	53.25	55.32	55.05	0.95
pH	5.05	5.25	5.55	5.85	5.65	5.75	5.45	5.35	5.85	0.66
Crude protein (%)	20.03	21.15	20.05	21.25	21.05	20.08	20.85	20.72	20.50	1.98

protein of the post-weaned growing rabbits.

Table 5 shows the interaction between feed restriction and period of restriction on meat quality of post- weaned rabbits. The interaction between levels of feed restriction and period of feed restriction had no significant ( $p>0.05$ ) effect on moisture, total cholesterol, pH and crude protein of the post-weaned growing rabbits.

## Discussion

Main effect of level of feed restriction and periods of feed restriction on carcass characteristics shows that there are no significant differences on carcass weight and dressing percentage. The result obtained in this study on carcass and dressing percentage is within the ranges of what was reported by Yakubu et al. (2007) in ad libitum fed rabbits. The similar values observed for head for the levels and periods of feed restriction is within the ranges of what was reported by Adejimi et al. (2013) in rabbits fed different fibrous ingredients. The values obtained for neck though significantly different, is within the ranges of values reported by Adejimi et al. (2013). The results obtained for forelimbs for the levels and periods of feed restriction is higher than what was reported by Adejimi et al. (2013). However, the mean values for hindlimbs for the levels and period of feed restriction obtained in this study is lower than what was reported by Adejimi et al. (2013) in rabbits fed different fibrous ingredients. The mean values obtained in this study for loin for the levels of feed restriction though significant is lower than what was reported by Tumova et al. (2006) in ad libitum fed rabbits. The result of carcass obtained for back in this study though significant for the levels of feed restriction and similar for the periods of feed restriction is higher than what was reported by Adejimi et al. (2013). The results obtained in this study for kidney, heart and spleen for the levels and periods of feed restriction is within the ranges of what was reported by Adejimi et al. (2013) in rabbit fed different fibrous feed ingredients. Interaction between levels of feed restriction

and periods of feed restriction on carcass characteristics shows that significant difference was obtained on carcass weight of the growing rabbits. The result obtained in this study across the levels and periods of feed restriction on carcass weight is slightly lower than what was reported by Yakubu et al. (2007) in ad libitum fed rabbits. The mean values obtained for the period and levels of feed restriction in this study for dressing percentage is higher than 53% that was reported by Yakubu et al. (2007). The mean values obtained in this study though significant for neck (2.37) and back (12.79) across the levels and period of feed restriction of does is higher than what was reported by Adejimi et al. (2013) who reported (1.40) for neck and (11.46) for back in rabbits fed diets containing fibrous ingredients. The values obtained in this study for hindlimb and loin though significant, is lower than what was recorded by Tumova et al. (2006) in ad libitum fed rabbits. The values obtained in this study though significant for lungs, heart, kidney and spleen across the levels and periods of feed restriction is within the ranges of what was reported by Adejimi et al. (2013) in rabbits fed diets containing fibrous ingredients.

Results obtained on main effect and interactive effect on moisture of post weaned growing rabbits though not significant is slightly lower than what was reported by Nistor et al. (2013) who reported moisture to be  $68.5 \pm 1.05\text{g}/100\text{g}$  and similar to what was reported by Apata et al., 2012 who reported moisture to be  $65.13 \pm 0.05$  in growing rabbits fed ad libitum diets. Results obtained on total cholesterol of post- weaned growing rabbits in this study varies across the dietary treatments though not significant is within the ranges of what was reported by Nistor et al. (2013) who reported cholesterol to be  $56.4 \pm 0.92 \text{ mg}/100\text{g}$  in rabbits fed ad libitum diets. pH values obtained for main effect and interactive effect in this study though not significant is within the ranges of values reported by Apata et al., 2012 who reported pH value to be  $5.85 \pm 0.05$  and Ragab et al., 2013 who reported pH value to be 5.50 in rabbits fed ad libitum diets.

## Conclusion

This study revealed that feed restriction during pregnancy resulted into higher carcass weight, dressing percentage and other cut up parts at the end of the sixteen week of the experiment. Feed restriction during pregnancy had no adverse effect on meat quality parameters of the growing rabbits measured as none of the parameters were affected by the treatment applied on the rabbit does during pregnancy. Thus feed restriction can be applied on pregnant does without any adverse effect on the meat quality of the post-weaned rabbits.

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## SERO-EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE IN SUDAN

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### Summary

The prevalence of antibodies against the three active infections of foot-and-mouth disease (FMD) in Sudan; "O", "A" and "SAT2", was studied in more than 1500 cattle sera, collected from 7 states in 2013, using a screening format of serum neutralization test (SNT). Prevalence's detected were 60.16%  $\pm$  2.25, 30.04%  $\pm$  2.19 and 12.31%  $\pm$  1.59 for type "O", "A" and "SAT2" respectively. The result indicated intense circulation and wide distribution of type "O" in the country as opposed to a likely significant introduction of type "SAT2" and "A". The geographical distribution of FMD infections was defined as penetrating along the Nile basin, particularly, for "O" and "A" viruses and more favorable at Eastern and Western Sudan. Still more favorable conditions are to be expected North of Khartoum and in Darfur in the far West. Type "O" was intensely disseminated along the Nile basin and distribution decreased away from there.

The Blue Nile state, at the South Eastern corner of the country, was an important route of entry of the three FMD viruses. Evidently, more risk factors were associated with intense traffic and movements of livestock related to national trade along and from the Nile basin than with movement of livestock associated with nomadic pastoralists in the East and West of the country. Nonetheless, movement of nomadic pastoralists in the East was associated with the potential risk of introduction of type "A" through the extended Eastern border of the country from Wad El Helew in Kassala to Aljabalein in the White Nile state.

**Keywords:** Foot-and-mouth disease; Sudan; epidemiology; Serum neutralization test; type "O", "A" and "SAT2" viruses

## SÉROÉPIDÉMIOLOGIE DE LA FIÈVRE APHTEUSE AU SOUDAN

### Résumé

La prévalence des anticorps contre les trois infections actives de fièvre aphteuse au Soudan, à savoir «O», «A» et «SAT2», a été étudié dans plus de 1500 sérums de bovins, prélevés dans 7 États en 2013, selon un mode de dépistage du test de neutralisation du sérum (SNT). Les taux de prévalence détectés étaient de 60,16%  $\pm$  2,25, 30,04%  $\pm$  2,19 et 12,31%  $\pm$  1,59, respectivement pour les types «O», «A» et «SAT2». Le résultat est une indication d'une circulation importante et d'une large distribution du type «O» dans le pays, par opposition à une introduction probablement significative de «SAT2» et «A». En ce qui concerne la répartition géographique des infections à fièvre aphteuse, il a été déterminé que son inclusion se faisait le long du bassin du Nil, en particulier pour les virus «O» et «A», les conditions étant plus propices à l'est et au sud-ouest du Soudan. Des conditions encore plus propices sont à prévoir au nord de Khartoum et au Darfour dans l'extrême-ouest. Le type «O» a été intensément disséminé le long du bassin du Nil et sa distribution a diminué à partir de cette région.

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L'État du Nil bleu, au sud-est du pays, a été une voie importante d'entrée des trois virus de la fièvre aphteuse. De toute évidence, un plus grand nombre de facteurs de risque ont été associés à une circulation intense et à des mouvements de bétail résultant du commerce national le long et à partir du bassin du Nil, plutôt qu'avec des mouvements de bétail appartenant aux pasteurs nomades à l'Est et à l'Ouest du pays. Néanmoins, le mouvement des pasteurs nomades à l'Est a été associé au risque potentiel d'introduction du type «A» à travers la longue frontière orientale du pays, qui s'étend de Wad El Helew à Kassala jusqu'à Aljabalein dans l'État du Nil Blanc.

**Mots-clés :** fièvre aphteuse, Soudan, épidémiologie, test de neutralisation de sérum; virus des types "O", "A" et "SAT2"

## Introduction

Foot-and-mouth disease (FMD) is the most important transboundary animal disease. It is caused by an Aphthovirus of which seven immunology distinct serotypes are known; "O", "A", "SAT 1, 2 and 3", "Asia 1" and "C". In most of Sub-Saharan Africa, four serotypes prevail; "O", "A", "SAT2" and "SAT1" (Vosloo *et al.*, 2002). In Sudan, at the upper Western corner of Eastern Africa, FMD was known to exist since 1903 (Abu Elzein, 1983) and the maintained activity of three serotypes has been lately confirmed; "O", "A" and "SAT2" (Raouf *et al.*, 2010; Habiela *et al.*, 2010: a). The fourth serotype, "SAT1", has not been reported in the country since 1976 and recently insignificant sero-prevalence was detected (Raouf *et al.*, 2009). In Sudan, cattle are the main target species and clinical FMD typed disease events were all of cattle origin (Abu Elzein, 1983; Habiela *et al.*, 2010: a; Raouf *et al.*, 2010).

In many parts of Africa, following the control of rinderpest (RP) in the early years of third millennium, more attention was directed to the problem of FMD. The FAO/OIE responded by developing the "progressive control pathway for control of FMD" (PCP-FMD) (FAO, 2011). The PCP-FMD, in its early stages, involves extensive surveillances in order to develop a working hypothesis of how FMD viruses are introduced and circulated in the country in an attempt to develop a risk-based control policy. Unlike RP, FMD is characterized by a complex epidemiology and an expensive control. Judicial vaccination against FMD in place of blanket vaccination such like that used against RP is much more in favor. Important elements of such a risk based control policy

are determining of hot spots, geographical distribution of FMD viruses and routes of introduction and circulation.

The presented work is an effort to determine these elements of a risk based control policy for FMD in Sudan. It was a FAO/Government of Sudan joint effort. A range of activities were assembled in a technical corporation programme (TCP/SUD/3401) to promote surveillances of FMD in the country. Sero-surveillance was one approach which was used extensively during the programme. Prevalence data, serotype and topotype distribution are the bases for a working hypothesis of how FMD introduced and circulated in an area (Rwemamu *et al.*, 2008). Appropriate number of sera examined by an appropriate serological test is likely to give the most accurate index of prevalence of a particular virus. Noteworthy, in likely FMD enzootics areas, subclinical and mild disease events are more expected. In this work, cattle sera from seven Sudanese states were examined for type specific antibodies; "O", "A" and "SAT2". Vaccination against FMD was not practiced to any appreciable extent in Sudan and positive serology was indicative of previous exposure.

## Materials and Methods

### Study area:

The study area included seven Sudanese states, North Kordfan, South Kordfan, White Nile, Blue Nile, Sinnar, Algdarif and Kassala (Fig. 1). It harbors about 50% of the country 30-40 millions head of cattle and constitutes about one third of the country area (1.6 million Km<sup>2</sup>). All studied states are border areas except

North Kordfan. Almost the whole of the study area falls in the low rainfall savannah except parts of North Kordfan and Kassala state which fall in the semi-desert ecological zone. In the savannah, cattle density is generally between 10 and 30 head per square Km and higher density of small ruminants, generally, between 25 and 100 head per square Km is expected. While < 5 cattle and < 10 small ruminants per square Km are expected in the semi-desert zone (FAO, 2005).

The study area could be 'clustered' in geography into 3 clusters; Western (North and South Kordfan states), South Eastern (Sinnar, White and Blue Nile states) and Eastern (Algdarif and Kassala states). The Western cluster is characterized by extensive pastoralism. Local cattle breeds in the Western cluster are mostly Baggara and constitute around 15% of the cattle population in the country. The South Eastern cluster shows the highest cattle density in the country containing nearly one third of the country cattle population. It is the Southern part of a larger geographical cluster; the Nile basin. The Eastern cluster showed the lowest cattle density in the study area; about 5% or lower of the country population.

#### *Sample size and serum collection:*

Tested sera were collected, in January 2013, from healthy cattle of one year old or above and with no history of vaccination against FMD. Simple random samples were collected from a sampling frame consisted of provinces/districts and collection sites respectively (table 1). A total of around 1900 cattle were sampled; distribution in different states is shown in table (1). The total number of cattle sampled in each state was determined using the formula described by Putt et al (1987):

$$N = \frac{P(100-P)}{SE^2}$$

Where

N= sample size

P= expected prevalence

SE= Standard error as an absolute accuracy

To maximize the number of cattle sampled,

the expected prevalence (P) was assumed to be 50%; the least favorable. It was decided that precision of 7% as an absolute accuracy (SE) would not affect our interpretation of results at the 95% confidence level. The obtained sample size (51), calculated in the mentioned formula, should be multiplied by 4 to compensate for error in random sampling from a wide geographical area (Putt et al, 1987). Where resources and information had permitted more than 200 animals were sampled from each state (table 1).

#### *Testing protocol:*

Sera were tested for serotype specific antibodies ("O", "A" and "SAT2") using a screening format (Raouf et al., 2012) of serum neutralization test (SNT). Tested sera were inactivated at 56 °C for 30 minutes, cooled, received about 5 µl penicillin/streptomycin mixture then, each, was divided into 2 or 3 aliquots and kept at -20 °C. Sera from different states were tested simultaneously i.e. each test plate included sera from 2 to 3 states.

#### *Screening format of SNT:*

##### *Viruses:*

FMD viruses used in the format were recent local isolates referred to by its serotype, geographical origin within Sudan, year and order of isolation from that origin as follow: O-Jaz 1/08, SAT2-Kh 2/08 (Raouf et al., 2010) and A-Kh 2/011. The first two viruses were isolated and adapted to grow in cultures of calf kidney cell (CKC) then received 3-4 passages in BHK 21 cells. Type "A" virus was isolated and adapted to grow in BHK 21 cells using information provided by the World Reference Laboratory (WRL) for FMD (Pirbright, UK). Viral cultural material was re-identified using IZSLER (Grazioli et al., 2012) antigen detection ELISA.

For the neutralization test, viruses were grown in BHK 21 cells, clarified by centrifugation at 2000 rpm for 10 minutes, distributed in 2 ml aliquots and stored in liquid nitrogen vapor. Virus stocks were titrated in the microtitre system as described before (Raouf et al., 2010) using BHK 21 cells. Virus

was diluted in complete Glasgo minimum essential medium (GMEM) {GMEM containing 10% tryptose phosphate broth (V/V), 0.0487% Na HCO<sub>3</sub> (W/V) and 10% (V/V) tris-buffer (0.05M)}. Growth media for BHK 21 cells contained in addition 10% (V/V) newborn calf serum (NBCS) (Sigma). Titres were calculated according to the method of Kärber (1931).

#### Control sera:

Positive control sera were homologous post-inoculation sera of O-Jaz 1/08, A-Kh 2/011 and heterologous post-inoculation sera of SAT2-Kh 1/08. Negative control serum was NBCS (Sigma). Control sera were inactivated in similar manner to test sera.

#### Procedure:

The procedure applied was as described before (Raouf *et al.*, 2012). Sera were tested at final dilutions of 1/32 (10-1.5) and 1/64 (10-1.8). Each serum was tested in 4 wells; 2 wells for each dilution. Each microtitre plate tested 24 or 20 test sera in addition to controls. Controls in each round of test comprised cell, virus, positive and negative serum controls; each of 4 wells. Serum diluent, virus diluent and growth media for BHK 21 were as described in virus titration.

In brief, serum dilutions were prepared in u-bottomed microtitre plates (Coaster) in 50 µl volume according to plan layout. Fifty µl of a previously titrated virus stock, containing 100 TCID<sub>50</sub>, was added to each plate well except the 4 wells of the cell control which received in place virus diluent. The virus control received no serum but serum diluent. Plates were sealed with adhesive tape, lightly tapped and left at room temperature for one hour. Fifty µl of suspension of BHK 21 cells, in growth medium, was added to each plate well.

Sealed plates were incubated at 37 °C with a source of humidity and read microscopically 2-3 days later. On the 3rd day post-seeding, plates were stained with 0.1% (W/V) crystal violet stain in 10% (V/V) formol-saline. Positive wells were stained (intact sheet) and negative wells were empty or with remnants of cells. The format detects

positive sera with a titre as low as 101.5 and demonstrates reproducibility in other 3 wells.

#### Statistical analysis:

Prevalence rates were determined by dividing the number of positive serum samples, identified by the tests, by the total number of samples tested in each sub-population. A 95% confidence interval (CI) from a simple random sample was derived using the formula described by Thrusfield (2005) for calculation of a confidence interval for a proportion; based on the Normal approximation to the binomial distribution:

$$P \pm 1.96 \sqrt{p(1-p)/n}$$

Where:

P= the estimated prevalence

n= number of samples tested

1.96= the appropriate multiplier for the selected confidence level

To determine whether prevalence rates of sub-population units were statistically significantly different, CI measures and P-values were used complementary (Du Prel *et al.*, 2009). The p-values were derived using a Z statistic to compare 2 proportions and a two-tailed test, available at the site (<http://epitools.ausvet.com.au/>). The CI measures were first compared; when CI values did not overlap then the statistic will always be statistically significantly different (StatNews # 73). For overlapping CI values, p-values were compared; results were significantly different, if  $p < 0.05$ . The p-values for CI values that did not overlap were calculated but not included.

## Results

#### Prevalence rates:

Type "O" showed the highest seroprevalence (60.16%) followed by type "A" (30.04%) then type "SAT2" (12.31%) (table 2). The order of FMD infections in different states remained similar; "O", "A" then "SAT2" (Fig. 2), yet; on the other hand, differences between states in type specific prevalence rates were evident (table 2).

### Identification of hot spots:

The Blue Nile state showed higher sero-prevalence, for the three serotypes, than most studied areas (table 2). In comparison to the White Nile state, it showed, beside, the higher sero-prevalence of type "SAT2", similarly high sero-prevalence's in all investigated districts/localities (Altdamn, Aldmazin, Bao) apart from one case; type "A" (13.3%) at Alroseirs district (n=30). It was identified as a hot spot. The identified hot spot could be extended to neighboring areas in the White Nile and Sinnar states which showed similarly high sero-prevalence's (Fig. 3).

### Geographical distribution of FMD infections:

The geographical cluster that represents the Nile basin (Blue Nile, White Nile and Sinnar) showed higher prevalence rates, for the 3 virus serotypes, than the Western (S. and N. Kordfan) and Eastern cluster (Algdarif and Kassala) (Fig. 4). The Eastern cluster showed lower sero-prevalence of type "O" and "SAT2", but not type "A", than the Western cluster (Fig. 4).

Comparing the South and the North fields of the country (Fig. 1), it was apparent that FMD infections did not simply follow a South North direction. Sero-prevalence of type "O" at N. Kordfan (67.49%) in Central Sudan was similar ( $P=0.0546$ ) to that at the Blue Nile (75.65%) and surpassed significantly ( $P=0.0001$ ) that at S. Kordfan (46.27%) in the South (table 2). Sero-prevalence of type "O" at Kassala (50.56%) in North Eastern Sudan was similar ( $P=0.3199$ ) to that at S. Kordfan (Table 2). The relatively high sero-prevalence of type "A" at Blue (41.62%) and White Nile (38.41%) states in the South was matched by that (32.62%) at Algdarif ( $P=0.0682$  and  $0.192$  respectively) in Eastern middle Sudan (Table 2). Merely, sero-prevalence of type "SAT2" at the Southern state of the Blue Nile (40.28%) was unmatched in Northern areas (Table 2).

### Entry and circulation of FMD viruses in Sudan:

Results presented in fig (5) shows a circle that surrounds the Upper Nile state of the Southern Sudan Republic (circle 2). All

border points investigated within this circle showed exceptionally high sero-prevalence of type "O" antibodies (between 75% and 89%) apart from one point (Aldali) at Sinnar state. No other border point in the six studied Border States showed similarly high sero-prevalence of type "O" (compare with fig. 8). The described circle could be identified as one possible route of entry of type "O" into Sudan.

Type "O" virus circulates intensely in the Nile basin states; Blue Nile, White Nile and Sinnar; and North Kordfan states as evident by high sero-prevalence rates (Table 2). The impact and significance of the within country circulation is shown in figures (6), (7) and (8). In Sinnar state (Fig. 6), sero-prevalence of type "O" detected (66.37%) in the Northern areas (East Sinnar and Sinnar) (n=113), neighboring Al Jazeera (not included in this study) and neighboring central or Northern areas of White Nile states was significantly higher ( $P=0.0052$ ) than that at the Eastern border area (Addinder) and the centre of the state (Singa) (42.55%) (n=47). It was higher than the Southern areas neighboring the hot spot of the Blue Nile state. In Algdarif (Fig. 6 and 8), significantly higher sero-prevalence of type "O" antibody (52.63%) was detected at areas neighboring Sinnar state (El Faw, El Ehad and Basunda) (n=76) rather than at border areas and at the centre of the state (30%) (El Fashaga, El Quresha, East El Qalabat and Middle Algdarif) (n=80) ( $p=0.0041$ ). Similarly, in Kassala state (Fig. 6 and 8), sero-prevalence of type "O" antibody at the border point of Wad El Helew (47%) was significantly lower than at areas within the state (Shagarab, Halfa El Gadeeda and Wagar) (68.75%) (n=64) that neighboring the main national road from Khartoum to Port Sudan ( $p=0.0371$ ). In Western Sudan (Fig. 7), likewise, significant higher prevalence of type "O" antibody in areas flanking the Nile basin rather than in Western areas was observable.

Type "SAT2" showed significant unmatched prevalence of 40.28% at the Blue Nile state (table 2). Being a border state, it was a likely route of introduction of this virus serotype into Sudan (Circle 1 in Fig 5). High sero-prevalence's for the virus serotype were

observable at neighboring areas in White Nile (Aljabalein) (Fig. 3) and in Sinnar state (Alsokie and Aldali) and, particularly, along the national road from the Blue Nile up to Abu Hujar and Singa (Fig. 6) in the latter state.

Type "A" showed the highest prevalence at the B. Nile (41.68%) and W. Nile (38.41%) which was statistically significantly different from all other states apart from Algdarif (32.62%) in the East (table 2). That was closely followed by prevalence in Sinnar state (29.44%); statistically similar to that in Algdarif ( $P=0.477$ ) but significantly lower from that in the B. Nile ( $P=0.0074$ ) and W. Nile ( $P=0.0262$ ) (Table 2). These 4 states represent the South Eastern and Eastern border of the country. When border points in the Eastern border were examined (Fig. 8), they showed significantly higher sero-prevalence of type "A" (Wad Alhelw in Kassala, Al quresh, E. al Qalabat and Basunda in algdarif) or demonstrated stronger effect (Aljabalien in W. Nile and Addinder in Sinnar) than neighboring areas within the country. Furthermore, only 4 localities inside the country (shown in fig. 8) showed similarly high significant values. Results suggested higher circulation than elsewhere and likely introduction of type "A" virus through these border areas.

## Discussion

It has long been known that type "O" is a predominant infection in Sudan (Abu Elzein, 1983; Abu Elzein *et al.*, 1987). However, what distinguished in this surveillance was the extent of type "O" predominance. It predominates the other two types of infection in all studied states (Fig. 2), unlike previous surveillances (Abu Elzein *et al.*, 1987; Habeila *et al.*, 2010;a; Raouf *et al.*, 2011). Such result was reflecting either true risk factors or insensitivity of type "A" and "SAT2" assays. The latter suggestion could largely be ruled out since both assays detected subtle differences between localities and regions, route of introduction and circulation of the two viruses in Sudan.

Not merely prevalence rates of the 3 virus types differed widely (60.16%, 30.04% and

12.31% for "O", "A" and "SAT2" respectively), but distribution of each serotype as indicated by prevalence indices paraded a different portrait. Type "O" circulated intensely inside the country whereas it showed less circulation in border areas apart from the circle shown in fig. (2). Type "A" showed more intense circulation in all studied border points (Fig. 8) than points within the country. Type "SAT2" showed significant prevalence merely in the B. Nile state and surrounding areas (Fig. 2). The result suggested an intense within country circulation of type "O" compared to less significant introduction from outside the country whereas introduction mechanisms of type "A" and "SAT2" into Sudan might play more significant role than their circulation within the country. A recent molecular study of FMD in Sudan (Habeila *et al.*, 2010;b), also, suggested that within-country circulation is an important mechanism by which type "O" is maintained in the country while detected recent representative of FMD isolates from other neighboring countries for lineages "A" and "SAT2". The result, also, gain support from the type-situation studies in Sudan in the last 8 years preceding the present surveillance. Type "O" virus was typed, almost, on yearly bases in 2005, 2007, 2008, 2010, 2011 and 2012 (Habeila *et al.*, 2010;a; Raouf *et al.*, 2010; Anon, 2010, 2011 and 2012) whereas type "A" was detected twice in 2006 and 2011 (Anon, 2006, 2011) and type "SAT2" was detected 4 times in the years 2005, 2007, 2008 (Habeila *et al.*, 2010;a; Raouf *et al.*, 2010) and lastly before 3 years in 2010 (Anon, 2010).

The presented work is the first trial to determine the geographical distribution of FMD infections in the country. Facts emerged have indicated higher distribution in the Nile basin than in Western and Eastern Sudan (Fig. 4). Outside the Nile basin, the highest prevalence for type "O", the most predominant and most wide spread in the country, was detected in North Kordfan (table 2). North Kordfan is a non-border state. It is the one state in the study area with the longest border area with the Nile basin (Fig. 1). The situation was consistent with previous findings that

indicated high distribution of FMD infections in cattle in Khartoum and Al Jazeera states along the River Nile and the Blue Nile respectively. Khartoum state was identified as hot spot and showed twice high sero-prevalence rates of 81%, 85% and 65% (Raouf *et al.*, 2011) and of 81%, 67% and 24% (FAO, 2014) for type “O”, “A” and “SAT2” respectively. Similarly, Al Jazeera state demonstrated high sero-prevalence’s of 71%, 82% and 41% for type “O”, “A” and “SAT2” respectively (Habiela *et al.*, 2010:a). These findings contradict a highly conventional believe that FMD infections in Sudan spread from the West and South to the East and North; following the pattern of animal movement. In this contest, it is interesting to observe how the prevalence of type “O”, the most widespread in Sudan, was significantly higher in North rather than in South Kordfan (table 2) and in Eastern (neighboring the Nile Basin) rather than in Western areas in the Western states of North and South Kordfan (Fig. 7). The geographical cluster of the Nile basin is distinguished by sedentary livestock animal production systems (sedentary irrigated-crop livestock animal production, the intra-urban backyard and the improved modernized systems) in comparison to nomadic pastoralist in Western and Eastern Sudan. The South-east area (White Nile, Blue Nile and Sinnar states) of the Nile basin showed the highest animal density in the country (FAO, 2005; Anon, 2009). In addition, the Nile basin is characterized by intense traffic and movement of animals associated with national trade. Cattle move to and fro from the animal markets in the Nile basin to the breeding areas in Western Sudan. Accordingly, it could be concluded that more risk is associated with traffic and movement of livestock along and from the Nile Basin than with movement of livestock associated with nomadic pastoralists in the West and East of the country.

The significance of the suggested introduction mechanism of type “A” through the Eastern border as opposed to its circulation inside the country should be emphasized. Like type “O” virus; type “A” has showed statistically significantly higher sero-prevalence

at the Nile basin cluster (B. Nile, W. Nile and Sinnar) than in the Western (S. and N. Kordfan) and Eastern (Kassala and Algdarif) clusters (Fig. 4). Moreover, in previous occasions, it has showed, along the Nile basin, similarly high sero-prevalence’s like those of type “O”, at Khartoum state, 85% (Raouf *et al.*, 2011) and 67% (FAO, 2014), and at Al Jazeera state, 82% (Habiela *et al.*, 2010:a); both states were not included in this work. Yet, whereas the highest prevalence of type “O” at the B. Nile was followed by that at the W. Nile ( $P=0.9658$ ) and N. Kordfan ( $p=0.0546$ ), the highest prevalence of type “A” at the B. Nile was followed by that at the W. Nile ( $P=0.47$ ) and Algdarif ( $P=0.0682$ ). The Western cluster showed statistically significantly higher sero-prevalence of type “O” (and even the least prevalent type “SAT2”), but not type “A”, than the Eastern cluster (Fig. 4). Type “A”, like type “O”, has long been known to be existing in Sudan. Nonetheless, it is well documented (Sutmöller and Vieira, 1980; Pay and Hingley, 1987) that following vaccination similar neutralization titres were associated with higher protection against homologous challenge in case of type “A” than in case of type “O”. Typing of “A” clinical disease within all reported period in Sudan (Abu Elzien, 1983; Vosloo *et al.*, 2002; Habiela *et al.* 2010; Raouf *et al.*, 2010) has been much less frequent than type “O” and in the last 8 years, preceding this work, even less frequent than type “SAT2”. It is to be expected that such mechanisms, had they been operating following natural infections, are likely to limit the significance of the within-country circulation of type “A”; but not circulation across the border. In the latter case the circulating virus would be sufficiently different to cause significant spread. On other words, repeated introductions might occur readily whereas an evolving virus inside the country needs more time to change sufficiently to cause a new outbreak.

The significance of the suggested introduction mechanism of type “SAT2” vs. the detected limited sero-prevalence inside the country was not unlikely. The virus type was the last to be recorded in Sudan in 1977 (Abu Elzein and Crowther, 1979). The detected

limited sero-prevalence, in this work in 2013, is not to be separated from a wide dissemination of type "SAT2" early in winter months in 2014 in 4 states; Khartoum, Al Jazeera, N. Kordfan and Algdarif (FAO, 2014). In this work, sero-prevalence of antibody to type "SAT2" was observed to rise along the national road "B. Nile-Khartoum" at Abu Hujar and Singa (Fig. 6) early in 2013. Late in April/2013 the virus type was detected in Khartoum state (FAO, 2014) then spread (phylogenetic distance < 5%) to the previously mentioned states early in 2014 (FAO, 2014; FAO WRLFMD, 2016).

A working hypothesis of how FMD is introduced and circulated in Sudan is emerging. The Blue Nile state and the extensions shown in fig (3) were important routes of entry of the three FMD viruses. Type "O" circulated intensely along the Nile basin and spread from there to Western and Eastern Sudan (Fig. 7 and 6) whereas introduction of type "SAT2" (Fig. 5) through the B. Nile state and type "A" (Fig. 8) through the extended Eastern border of the country from Wad Al Helew in Kassala down to Aljabalein in the White Nile state into the country are significant. Accordingly, in Western Sudan, little introduction from Darfur border States is likely predictable whereas in Northern Sudan where limited animal population (FAO, 2005; Anon, 2009) is known, less circulation of FMD infections could be expected. More efforts are required to define other possible mechanisms of introduction and circulation. Traceability by molecular studies could lend additional valuable information to patterns of circulation and introduction in the country.

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## **OCCURRENCE OF SOIL-TRANSMITTED HELMINTHS ON PLAYGROUNDS OF NURSERY AND PRIMARY SCHOOLS AND ASSOCIATED RISK FACTORS IN PLATEAU STATE, NIGERIA**

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### **Abstract**

The highest magnitude of soil transmitted helminths documented in sub-Saharan Africa in recent years occurred in Nigeria. In this study, the occurrence of STHs on play grounds of nursery and primary schools and associated risk factors for environmental contamination in Plateau State were determined using conventional parasitological techniques and well structured close ended questionnaires. Data were analyzed using Chi square, odds ratio and logistic regression at 95% confidence interval. Of the 350 soil samples examined, 124 (35.4%) were positive for *Ancylostoma duodenale* (4.3%), *Ascaridia galli* (6.6%), *Ascaris lumbricoides* (5.4%), *Strogyloides stercoralis* (8.3%) and *Trichuris trichura* (10.3%). The distributions of STHs in relation to school categories were 9.1% (32/350), 22.3% (78/350) and 4.0% (14/350) for nursery, primary and combined schools respectively. The distributions of STHs across private, public, fenced, unfenced schools and LGAs varied significantly ( $p < 0.05$ ) and ranged between 22.2% and 50.8%. Open defecation was the most significant of all the risk factors that positively influenced the occurrence of STHs on play grounds. STHs are prevalent on play grounds of nursery and primary schools in Plateau State. Improved hygiene and sanitation, fencing of school premises and the regulation of school population will help to reduce environmental contamination and human infections.

## **PRÉSENCE D'HELMINTHES TRANSMIS PAR LE SOL SUR LES TERRAINS DE JEUX DES ÉCOLES MATERNELLES ET PRIMAIRES ET FACTEURS DE RISQUE ASSOCIÉS DANS L'ÉTAT DU PLATEAU AU NIGERIA**

### **Résumé**

La plus forte prévalence des helminthes transmis par le sol (HTS) documentée en Afrique subsaharienne au cours des dernières années a été enregistrée au Nigeria. Cette étude a déterminé la présence d'HTS sur les terrains de jeu des écoles maternelles et primaires et les facteurs de risque associés à la contamination de l'environnement dans l'État du Plateau, en utilisant des techniques parasitologiques classiques et des questionnaires orientés bien structurés. Les données ont été analysées à l'aide du Chi carré, du rapport de cotes et de la régression logistique à un intervalle de confiance de 95%. Des 350 échantillons de sol examinés, 124 (35,4%) se sont révélés positifs pour *Ancylostoma duodenale* (4,3%), *Ascaridia galli* (6,6%), *Ascaris lumbricoides* (5,4%), *Strogyloides stercoralis* (8,3%) et *Trichuris trichura* (10,3%). Les distributions des HTS par rapport aux catégories scolaires étaient respectivement de 9,1% (32/350), 22,3% (78/350) et 4,0% (14/350) respectivement pour les écoles maternelles, primaires et combinées. Les distributions des HTS dans les écoles privées, publiques, clôturées, non délimitées et les LGA varient considérablement ( $p < 0,05$ ) et se situent entre 22,2% et 50,8%. La défécation à ciel ouvert était le facteur de risque le plus significatif qui a significativement influencé la présence de HTS sur les terrains de jeu. Les HTS sont répandues sur les terrains de jeu des écoles maternelles et primaires dans l'État du Plateau. L'amélioration de l'hygiène et de l'assainissement, la construction de clôtures autour des locaux scolaires et la régulation de la population scolaire contribueront à réduire la contamination de l'environnement et les infections humaines.

## Introduction

Soil transmitted helminths (STHs) or geohelminths are a group of intestinal helminths that utilize soil as vehicles for their transmission. The five major zoonotic geohelminths include; the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichura*, the threadworm *Strongyloides stercoralis* as well as the hookworms *Ancylostoma duodenale* and *Necator americanus* which are all grouped under the class nematode and phylum nemathelminthes (Brooker *et al.*, 2006). They are worldwide in distribution and are the most prevalent and persistent of all infections in children. Ingestion of eggs and skin penetration by infective larval stages from environments contaminated with faeces of infected individuals are predominantly the major means of transmission of STHs (Greenland *et al.*, 2015).

Substantive evidence shows that majority of the over two billion people infected with STHs are children. Majority of geohelminth infections occur in sub-Saharan Africa where over 89 million children of school-age are infected (Brooker *et al.*, 2006). It was estimated that of the over one billion people infected with STHs, 450 million have significant morbidity attributable to the infection, 44 million are pregnant women and suffers from hookworm associated anaemia that contribute to maternal mortality and approximately 35, 000 dies of these infections yearly (Awasthi and Bundy, 2007).

Geohelminth infections are most prevalent in tropical and subtropical regions of resource-limited countries especially among the poor populations living in warm moist climates. Infections are influenced by urbanization which usually results in overcrowding and poor sanitation in developing countries and lack of anthelmintic treatment programmes targeting school-age children (Cooper, 2009). Other factors that may contribute to the transmission of STHs may include open defecation, uncontrolled scavenging animals, poverty as well as poor hygiene and sanitation (Ojurongbe *et al.*, 2014). Though mortality is

uncommon, heavy untreated infections may lead to malnutrition, anaemia, retarded growth and cognitive impairment (WHO, 1987; Callender *et al.*, 1992; Levav *et al.*, 1995).

Several studies in Nigeria documented varying prevalence rates of geohelminths ranging from 9.9 to 51.3% among pre-school age children (Meremikwe *et al.*, 2000; RunseweAbiodun and Olowu, 2009; Omitola *et al.*, 2016), 25.3 to 87.7% among school age children (Chukwuma *et al.*, 2009; Shehu *et al.*, 2013; Ojurongbe *et al.*, 2014), 1.0 to 43.4% among pregnant women (Egwunyenga *et al.*, 2001; Alli *et al.*, 2011) and 40.4 to 55.0% on vegetables (Elom *et al.*, 2012; Uneke *et al.*, 2015). However, there is paucity of information on the level of environmental contamination by STHs which is believed to be the source of human infection. This study was designed to bridge the existing gap and determine the occurrence of STHs on play grounds of Nursery and Primary Schools and associated risk factors in Plateau State, Nigeria.

## Materials and Methods

### Study area

Plateau State is located in North-central Nigeria and covers a land mass of 26,899 square kilometres. The population of the state was estimated at 3.5 million (Census, 2006). The state is located between latitude 8°24'N and longitudes 8°32' and 10°38'E (Figure 1). The altitude ranges from around 1,200 meters to 1,829 meters above sea level. Though situated in the tropical zone, the higher altitude gives the state a near temperate climate with average temperature ranging between 18 and 22 °C. The lowest temperatures are observed between December and February while the warmest temperatures usually occur between March and April. It has two distinct seasons namely; rainy which extends from May to October and dry which extends from November to April with average annual precipitations ranging from 1314.8 mm in the southern part to 1465.7 in the northern part and highest rainfall is recorded during July and August. The mean relative humidity varies between 14% and 74%

and the major occupation of inhabitants of the state is agriculture.

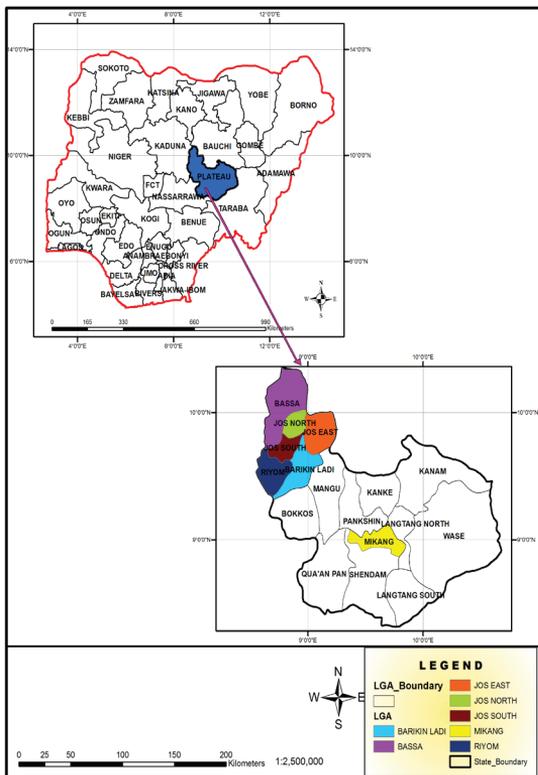
**Study design**

The study was a randomised cross section study. Using simple balloting, seven Local Government Areas (LGAs) were selected across the state and 175 nursery and primary schools were also selected within the LGAs. Sample size of 326.9 was determined using the formula of Thrushfield (1997), with an expected prevalence of 82.63% reported by Eke et al. (2015) and 95% confidence interval. Soil samples from any two different strategic locations within the pupils play grounds were collected giving a total of 350 soil samples. The distributions of samples within the sampled LGAs were based on the number of nursery and primary schools as shown in Figure 2. A total of 175 well structured close ended questions were administered to one teacher in each of the schools sampled to assess risk

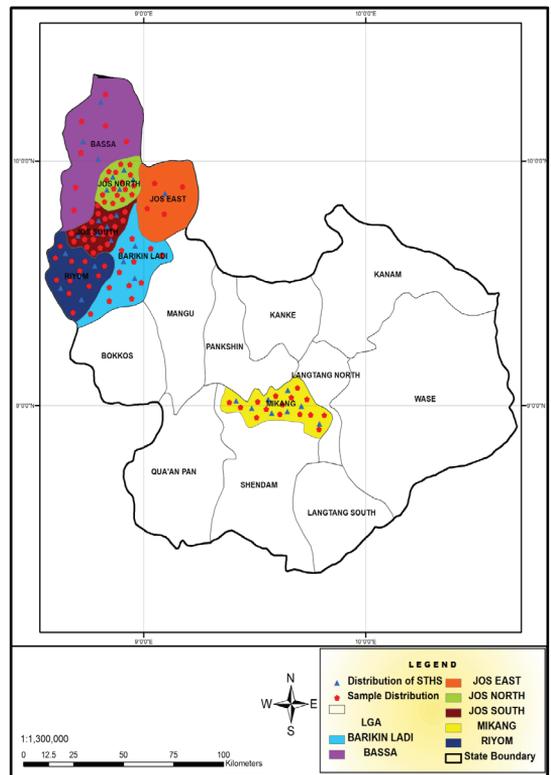
factors associated with the occurrence of STHs on play grounds of nursery and primary schools in Plateau State.

**Sample collection and laboratory analysis**

A total of 350 soil samples were collected in the morning hours between 8:00 and 10:00 am. A large portion of soil was pooled together, mixed thoroughly and 100 grams was collected thereafter into sterile containers for the analysis. These samples were transported to the Parasitology Laboratory of the National Veterinary Research Institute, Vom, Nigeria for parasitological analysis. Soil samples were suspended in saturated salt solution in the laboratory and sieved. Thereafter, the filtrate was processed using simple floatation and concentration techniques and eggs as well as larvae of STHs were identified using morphological characteristics as earlier described (Soulsby, 1982; Zajac and Conboy, 2006).



**Figure 1:** Map of Nigeria and Plateau State showing locations where samples were collected



**Figure 2:** Distributions of samples and STHs across the studied locations

### Data analysis

Data generated were analysed using Statistical Package for Social Sciences (SPSS Version 20.0) and Graph Pad Prism Version 4.0. Environmental prevalence of STHs was determined by dividing the number of soil samples contaminated with the total number of soil samples analysed and expressed as percentages. This was done for different variables such as species of STHs, school type and study sites. The Chi square ( $\chi^2$ ) test and odds ratio were used where appropriate to measure statistical associations between different variables while logistic regression was employed to determine statistical association between risk factors and environmental contamination by STHs and values of  $p < 0.05$  were considered significant.

### Results

A total of 350 soil samples from playgrounds of nursery and primary were examined for the presence of soil transmitted helminths in Plateau State, Nigeria. Of this number, 124 representing 35.4% were positive for different STHs. The distribution of STHs on playgrounds of schools were 4.3%, 6.6%, 5.4%, 8.3% and 10.3% for *Ancylostoma duodenale*, *Ascaridia galli*, *Ascaris lumbricoides*, *Stroglyoides stercoralis* and *Trichuris trichura* respectively. STHs distributions in relation to school category were 9.1% (32/350), 22.3% (78/350) and 4.0% (14/350) for nursery, primary and combined schools respectively (Table 1).

There was significant variation ( $p < 0.0001$ ,  $\chi^2 = 16.88$ , OR = 0.3939, 95% CI = 0.2512-0.6174) between the 27.8% (49/190) and 46.9% (75/160) of STHs distributed across private and public schools respectively. The distribution of STHs across fenced 22.2% (37/168) and unfenced 47.8% (87/182) schools also varied significantly ( $p < 0.0001$ ,  $\chi^2 = 25.38$ , OR = 0.3084, 95% CI = 0.1934-0.4919) as in Table 2. STHs were also significantly distributed ( $p = 0.0128$ ,  $\chi^2 = 16.19$ ,  $df = 6$ ) across Bassa (37.9%), Barkin Ladi (22.5%), Jos East (33.3%), Jos North (34.9%), Jos South (28.0%), Mikang (50.8%) and Riyom (40.4%) LGAs as in Table 3. The risks of contamination of play grounds of

nursery and primary schools in Plateau State were influenced by factors including open defecation ( $p < 0.0001$ , OR = 5.772, 95% CI = 3.829-8.700), the use of latrines ( $p < 0.0001$ , OR = 2.374, 95% CI = 1.639-3.439) and lack of fencing of school premises ( $p = 0.0003$ , OR = 1.979, 95% CI = 1.366-2.855) as shown in Table 4. Other factors such as intrusion of passers-bys ( $p = 0.0004$ , OR = 1.930, 95% CI = 1.335-2.790) and scavenging animals ( $p = 0.0007$ , OR = 1.886, 95% CI = 1.305-2.727) into school premises and overcrowding ( $p < 0.0001$ , OR = 2.320, 95% CI = 1.602-3.359) were also positively associated with the occurrence of STHs on play grounds of nursery and primary schools (Table 4).

### Discussion

Prevalence studies on geo-helminths infections are well documented in Nigeria especially among school age children. However, studies targeting sources of these human infections are lacking. This study therefore became necessary to provide additional information on the epidemiology of these neglected tropical parasitic infections in Plateau State, Nigeria especially with documented evidence showing the global public health threats pose by these infections among approximately 2 billion people particularly in sub-Saharan Africa, Americas, China and East Asia (Brooker *et al.*, 2006). The detection of these parasites on playgrounds of nursery and primary schools in Plateau State may suggest the possible source of the geo-helminth infections well documented among school age children in the region considering the poor hand hygiene practiced among children within this age brackets (Odebunmi *et al.*, 2007; Bala *et al.*, 2010).

The overall contamination levels of 35.4% observed by the present study is slightly higher than the 30.7% (Nwoke *et al.*, 2013) and grossly lower than the 82.6% (Eke *et al.*, 2015) documented respectively in Abuja and Ebonyi State, Nigeria. These variations may be due to differences in the levels of sanitation, environmental conditions

**Table 1:** Distribution of soil-transmitted helminths in relation to category of school.

Geohelminth species	Number positive (n = 350)	Overall Prev. (%)	Nursery (%) (n = 142)	Primary (%) (n = 160)	CB (%) (n = 48)
<i>Ancylostoma duodenale</i>	15	4.3	8 (5.6)	3 (1.9)	4 (8.3)
<i>Ascaridia galli</i>	23	6.6	5 (3.5)	16 (10.0)	2 (4.2)
<i>Ascaris lumbricoides</i>	19	5.4	6 (4.2)	6 (3.8)	7 (14.6)
<i>Strongyloides stecoralis</i>	29	8.3	8 (5.6)	15 (9.4)	6 (12.5)
<i>Trichuris trichura</i>	38	10.8	11 (7.8)	21 (13.2)	6 (12.5)
<b>Total</b>	<b>124</b>	<b>35.4</b>	<b>38 (26.7)</b>	<b>61 (38.2)</b>	<b>25 (52.1)</b>

CS (Combined schools)

**Table 2:** Distribution of soil-transmitted helminths in relation to school-type and availability or absence of fence

Variable	Number examined	Number positive	Prevalence (%)	p-value ( $\chi^2$ )	Odds ratio (95% CI)
<b>School type</b>					
Private	190	49	27.8	<0.0001	0.3939
Public	160	75	46.9	(16.88)	(0.2512-0.6174)
<b>Total</b>	<b>350</b>	<b>124</b>	<b>35.4</b>	-	-
<b>Fenced/Unfenced</b>					
Fenced	168	37	22.0	<0.0001	0.3084
Unfenced	182	87	47.8	(25.38)	(0.1934-0.4919)
<b>Total</b>	<b>350</b>	<b>124</b>	<b>35.4</b>	-	-

**Table 3:** Distribution of soil-transmitted helminths in relation to study sites.

Study sites	Number examined	Number positive	Prevalence (%)	p-value, df* ( $\chi^2$ )
Bassa	28	11	39.3	
Barkin Ladi	48	11	22.9	0.0128
Jos East	16	5	31.3	
Jos North	64	22	34.4	6*
Jos South	84	23	27.4	
Mikang	60	33	55.0	(16.19)
Riyom	50	19	38.0	
<b>Total</b>	<b>350</b>	<b>124</b>	<b>35.4</b>	-

including temperature, humidity, soil moisture and human activities in the various study areas. Human related factors including lack of public education (Ocathain *et al.*, 2002), lack of strategic deworming (WHO, 2002), season of study (Brooker and Michael, 2007), poverty and urbanization (Crompton and Saviola, 1993) as well as human behaviour and overcrowding

(Brooker *et al.*, 2004) may be other possible reasons for the environmental contamination. The species of geo-helminths identified by this study were earlier reported in Eastern (Nwoke *et al.*, 2013; Odinaka *et al.*, 2015), Southern (Adedike *et al.*, 2014; Omitola *et al.*, 2016) and Northern (Bala *et al.*, 2010; Eke *et al.*, 2015) Nigeria indicating that they are endemic

**Table 4:** Logistic regression of risk factors associated with occurrence of STHs in Plateau State.

Variables	NQA	TR	NR	P-value	Odds ratio	95% CI
Practice of open Defecation	175	Yes	133	<0.0001	5.772	(3.829-8.700)
		No	42			
Use of latrines	175	Yes	99	<0.0001	2.374	(1.639-3.439)
		No	76			
Lack of fence	175	Yes	91	0.0003	1.974	(1.366-2.855)
		No	84			
Intrusion of scavenging animals	175	Yes	89	0.0007	1.886	(1.305-2.727)
		No	86			
Intrusion of Passer-bys	175	Yes	90	0.0004	1.930	(1.335-2.790)
		No	85			
Overcrowding	175	Yes	98	<0.0001	2.320	(1.602-3.359)
		No	77			

NQA (Number of questionnaire administered), TR (Type of response), NR (Number of respondents)

in the country. These same species were also reported in other parts of the world (Debalke *et al.*, 2013; Sanchez *et al.*, 2013; Greenland *et al.*, 2015) suggesting also that they are a global problem. This finding also supports the report that Nigeria has the highest magnitude of STH infections in Africa (Brooker *et al.*, 2006).

The percentage distribution of *Trichuris trichura* (10.3%), *Strongyloides stercoralis* (8.3%), *Ascaris lumbricoides* (5.4%) and *Ancylostoma duodenale* (4.3%) are all within the ranges of 4.7-11.1%, 5.7-13.1%, 8.6-26.7% and 6.3-25.9% previously documented respectively in Nigeria (Adedike *et al.*, 2014; Odinaka *et al.*, 2015; Omitola *et al.*, 2016). This suggests persistence in the occurrence of these parasites, possibly due to problems with current control programmes that lead to their being shortlisted among neglected tropical diseases. Environmental conditions as well as poor sanitary practices including indiscriminate and open defecation by humans and animals may be possible explanations for the persistence of these parasites in the studied schools.

It is believed that the eggs of geohelminths are carried under shoes to these play grounds by children, and so the higher contamination levels observed in primary as compared to nursery schools may not be unconnected with the trekking of primary

school pupils to school as compared to nursery school pupils who are usually transported to school in vehicles. The higher contamination levels observed among public schools may not be unconnected with the intrusion by passer-bys and scavenging animals into their premises which are mostly unfenced. This may also explain the higher contamination levels observed among unfenced schools. The lower contamination levels observed among private schools may be due to the secured fences provided around most of these schools. It was also not surprising to have observed higher contamination levels in Mikang and Riyom which are rural settlements where people usually defecate indiscriminately as result of lack of toilet facilities.

The epidemiological and public health implications of this finding are the risks of nursery and primary school children acquiring STH infections from the contaminated environment and the possible transfer of these parasites and larvae to different locations either by rain water or shoes thus contaminating other environments that might as well result in human infections. The risk of intellectual and cognitive retardation, malnutrition and stunted growth that are possible outcomes of STH infections are also of great public health concern.

Open defecation was found to be the greatest risk factor associated with the occurrence of STHs on play grounds and was seen to influence contamination of play grounds 5.772 (3.829-8.700) times. This finding was not strange since most of these parasites are located within the gastrointestinal tract and transmitted via the faeco-oral route. The study also revealed the use of latrines to influence the risk of play grounds contamination by 2.374 (1.639-3.439) times. Practical experience has shown rain waters flooding latrines and contaminating the environment. This may be a possible explanation for the positive association between this risk factor and the occurrence of STHs on play grounds. Overcrowding in schools also influenced the occurrence of STHs on play grounds of schools about 2.320 (1.602-3.359) times. This was probably due to open defecation as a result of inadequate toilet facilities.

Lack of fencing school premises and intrusion of passer-bys and scavenging animals into school premises are all related and influenced the occurrence of these parasites on play grounds 1.974 (1.366-2.855), 1.930 (1.335-2.790) and 1.886 (1.305-2.727) times respectively. The intrusion of strangers and scavenging animals into school premises are all as a result of lack of fencing. These passer-bys may carry the eggs and larvae of these parasites under their shoes from other locations and deposit them on these play grounds resulting in environmental contamination. Open defecation by these scavenging animals may be another possible reason for the occurrence of these parasites.

### Conclusion

Play grounds of nursery and primary schools in Plateau State are contaminated with STHs. Contamination levels were higher in primary schools, public and unfenced schools and the schools located in rural parts of the State. Environmental contamination by STHs was highly influenced by open defecation. Other risk factors such as the use of latrines, lack of securing school premises, overcrowding

as well as intrusion of passer-bys and scavenging animals into school premises all influenced the occurrence of STHs on play grounds of nursery and primary schools in Plateau State. Improved hygiene and sanitation, securing school premises and the control of school population will reduce the risk of environmental contamination in Plateau State.

### Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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## EVALUATION OF NUTRITIVE VALUE OF WATER HYACINTH (EICHHORNIA CRASSIPES) AND GUINEA GRASS (PANICUM MAXIMUM) MIXTURE AS ANIMAL FEED IN THE TROPICS

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### Abstract

The nutritive value of water hyacinth (*Eichhornia crassipes*) and Guinea grass (*Panicum maximum*) mixture as animal feed resources in the tropics were evaluated. The mixture of the treatments: A (0% water hyacinth + 90% Guinea grass + 10% concentrate), B (30% water hyacinth + 60% Guinea grass + 10% concentrate), C (45% water hyacinth + 45% Guinea grass + 10% concentrate), D (60% water hyacinth + 30% Guinea grass + 10% concentrate) and E (90% water hyacinth + 0% Guinea grass + 10% concentrate) were incubated *in vitro* for 96 hours to monitor total gas production (ml/200 mg DM) at post incubation. Methane (mmol/200mg DM) was evaluated by introducing 10 M NaOH into the content. Metabolizable energy (MJ/Kg DM), organic matter digestibility (%) and Short chain fatty acids (mmol) were calculated. Results showed that the crude protein and neutral detergent fibre (g/100 g DM) ranged from 10.28 to 10.42 and 55.62 to 56.29, respectively. Inclusion levels of water weed apparently reduced the total gas production. It was concluded that water hyacinth as forage may be a good combination with Guinea grass for livestock production, but must not be higher than 30 % inclusion for optimal performance.

**Key words:** Guinea grass, *in vitro* gas fermentation, Ruminants, Secondary metabolites, Water hyacinth,

## ÉVALUATION DE LA VALEUR NUTRITIVE DU MÉLANGE DE JACINTHE D'EAU (EICHHORNIA CRASSIPES) ET D'HERBE DE GUINÉE (PANICUM MAXIMUM) COMME ALIMENT POUR ANIMAUX EN MILIEU TROPICAL

### Résumé

La présente étude a évalué la valeur nutritive du mélange de jacinthe d'eau (*Eichhornia crassipes*) et d'herbe de Guinée (*Panicum maximum*) en tant que ressource alimentaire pour animaux dans les tropiques. Les mélanges des ingrédients aux diverses proportions, à savoir A (0% jacinthe d'eau + 90% Guinée + 10% concentré), B (30% jacinthe d'eau + 60% Guinée + 10% concentré), C (45% jacinthe d'eau + 45% Guinée (60% de jacinthe d'eau + 30% d'herbe de Guinée + 10% de concentré) et E (90% de jacinthe d'eau + 0% d'herbe de Guinée + 10% de concentré) ont été incubés *in vitro* pendant 96 heures dans le but de surveiller la production totale de gaz (ml / 200 mg de DM) après incubation. Le méthane (mmol / 200 mg de DM) a été évalué par introduction du NaOH 10 M dans le mélange. L'énergie métabolisable (MJ / Kg DM), la digestibilité de la matière organique (%) et les acides gras à chaîne courte (mmol) ont été étudiés. Les résultats ont montré que la protéine brute et la fibre détergente neutre (g / 100 g DM) variaient respectivement de 10,28 à 10,42 et de 55,62 à 56,29. Les niveaux d'inclusion de la mauvaise herbe aquatique ont apparemment réduit la production totale de gaz. Il a été conclu que la jacinthe d'eau peut constituer, en combinaison appropriée avec l'herbe de Guinée, une bonne ressource fourragère pour l'élevage, mais pour une performance optimale, son inclusion ne doit pas être supérieure à 30%.

**Mots-clés :** herbe de Guinée, fermentation de gaz *in vitro*, ruminants, métabolites secondaires, jacinthe d'eau

## Introduction

The low productivity of ruminant livestock is as a result of the poor nutritional status of forages in terms of quality (Otchere *et al.*, 1987). Lamidi *et al.* (1997) reported that inadequate feed provision especially during the 5-7 months dry season is one of the major limitations to ruminant livestock production in the Nigerian savanna rangelands. The native pastures deteriorates rapidly especially in the dry season. One of those in use is Guinea grass (*Panicum maximum*).

Guinea grass (GG) is one of the most common grasses in the derived savannah region of Nigeria. Under good conditions, its nutritional value is high; having up to 12.5% crude protein (CP), total digestible nutrients (TDN) of 10.2% and calcium, phosphorus and magnesium. (McDonald *et al.*, 1988). Pastures can be supplemented with other alternative fodder such as aquatic plants, the use of aquatic plants as fodder in Nigeria is still in its infancy. Water hyacinth is the most common water weed in Nigeria being in abundance during the dry season (Akinyemiju 1987). It is rich in nutrients with a crude protein of 14.40 % in leaves (Dairo 1999), 10.20 % in leaves plus stems (Dada 2002). Omojola *et al.* (2001) reported a crude protein of 11.34 % for water fern. A sustainable way of improving the feeding value of poor quality pastures is through supplementation (Bamikole and Babayemi, 2004).

The *in vitro* gas method is a laboratory estimation of important degradable feeds in livestock nutrition. It is a method that is reproducible and parameters obtained correlates well with *in vivo* method. *In vitro* gas method have the advantage of not only being less expensive and less time consuming, but also allows experimental conditions to be maintained more precisely than the *in vivo* method (Makkar 2002). It is convenient and fast and allows a large number of samples to be handled at a time. It is based on the quantification of substrate degraded and of gas or short chain fatty acids produced in rumen fermentation system based on syringes (Menke

*et al.* 1979).

This study was aimed at evaluating the chemical composition, secondary metabolites and *in vitro* gas production characteristics of water hyacinth shoot (leaf and stem) with guinea grass and concentrate.

## Material and Methods

### *Collection of samples*

Water hyacinth (*Eichhornia crassipes*) were collected in the months of May, August, November and February to coincide with early rain, late rain, early dry and late dry seasons, respectively. Twenty five (25) stands were harvested randomly in each season, while harvesting was done manually. Guinea grass was harvested during the rainy season. The harvested samples of Guinea grass and water hyacinth were washed thoroughly and air dried. A known sample weight of each sample was oven dried at 105 °C until constant weight for dry matter determination was attained (AOAC, 1995). The samples were thoroughly mixed and sub sampled. The dried samples were milled in a Thomas Willey laboratory mill fitted with 0.5 mm mesh. The milled samples were kept in air tight bottles until they were needed for chemical analysis.

### *Concentrate diet*

The composition of the concentrates is shown in Table 2 and was formulated to include all necessary nutrients required by goats.

### *Proximate composition*

Crude protein, crude fibre, ether extract and total ash of samples were analysed in triplicates using standard procedure of AOAC (1995). The crude protein was determined with the micro Kjeldahl distillation apparatus, while acid detergent fibre, was determined by Van Soest method (1994).

### *Quantitative determination of tannin, phenol and saponin*

Tannin contents were determined as described by Swain (1979). 0.20g of sample was measured into a 50ml beaker 20mL of 50%

methanol was added and covered with parafilm and placed in a water bath at 77-80°C for 1 hour. It was shaking thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No 41 filter paper into a 100ml volumetric flask, 20mL water added, 2.5ml folin-Denis reagent and 10ml of 17% Na<sub>2</sub>CO<sub>3</sub> were added and mixed properly. The mixture was made up to mark with water mixed well and allow to stand for 20 min. The bluish –green color will develop at the end of range 0-10ppm were treated similarly as 1mL sample above. The absorbances of the Tannic acid standard solutions as well as samples were read after color development on a spectronic 21D spectrophotometer at a wavelength of 760nm. Percentage tannin was calculated using the formula:

$$\frac{\text{absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10,000}$$

Phenol contents were determined as described by AOAC (1984). 0.20g of sample was weighed into a 50mL beaker, 20mL of acetone was added and homogenize properly for 1hr to prevent lumping .The mixture was filtered through a Whatman No.1 filter paper into a 100mL Volumetric Flask using acetone to rinse and made up to mark with distilled water with thorough mixing. 1mL of sample extract was pipetted into 50mL Volumetric flask, 20mL water added, 3mL of phosphomolybdic acid added followed by the addition of 5mL of 23% NaCO<sub>3</sub> and mixed thoroughly, made up to mark with distilled water and allowed to stand for 10min to develop bluish-green colour. Standard Phenol of concentration range 0-10mg/mL were prepared from 100mg/L stock Phenol solution from Sigma-Aldrich chemicals, U.S.A. The absorbances of sample as well as that of standard concentrations of Phenol were read on a Digital Spectrophotometer at a wavelength of 510nm. The percentage phenol is calculated using the formula:

$$\frac{\text{absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10,000}$$

The Spectrophotometric method of Brunner (1984) was used for saponin Analysis. 1g of finely ground sample was weighed into a 250mL beaker and 100mL of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing . Thereafter the mixture was filtered through a whatman No1 filter paper into a 100mL beaker and 20mL of 40% saturated solution of maganesium carbonate was added. The mixture obtained with saturated MgCO<sub>3</sub> was again filtered through a Whatman No1 filter paper to obtain a clear colorless solution. 1mL of the colorless solution was pipetted into 50ml volumetric flask and 2mL of 5% FeCl<sub>3</sub> solution was added and made up to mark with distilled water. It was allowed to stand for 30min for blood red color to develop. 0-10ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2mL of 5% FeCL<sub>3</sub> solution as done for 1mL sample above. The absorbance of the sample as well as standard saponin solutions were read after color development in a Jenway V6300 Spectrophotometer at a wavelength of 380min. percentage saponin was calculated using the formula:

$$\frac{\text{absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10,000}$$

#### *In vitro gas production method*

Water hyacinth samples collected during the four sub seasons were pooled and sub-samples for further analysis. The treatments were A (0% water hyacinth + 90% Guinea grass + 10% concentrate), B(30% water hyacinth + 60% Guinea grass + 10% concentrate), C (45% water hyacinth + 45% Guinea grass + 10% concentrate), D (60% water hyacinth + 30% Guinea grass + 10% concentrate) and E(90% water hyacinth + 0% guinea grass + 10% concentrate). Rumen fluid was collected from three West African dwarf female goats through suction tube before the morning feed. The animals were fed concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soya bean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster

**Table 1:** Proximate composition (g /100 g DM) of Water hyacinth shoot, Guinea grass and concentrate

	Water hyacinth (season)				Grass	Conc
	ER	LR	ED	LD		
Dry matter	7.63	7.63	7.93	8.22	31.21	89.78
Crude protein	10.28	10.38	10.38	10.42	7.28	18.12
Ether extract	1.54	1.55	1.55	1.56	3.20	3.88
Ash	19.94	20.31	20.53	20.54	6.59	7.63
Neutral detergent fibre	55.62	55.88	56.02	56.29	70.01	39.69

Season = ER= early rain, LR= late rain, ED=early dry, LD= late dry

Conc= concentrate,

**Table 2:** Ingredient composition (Kg/100 Kg DM) of concentrate fed WAD goats

Ingredients	Percentage composition
Maize	20.00
Wheat offal	25.00
Corn offal	18.00
Soya bean meal	8.00
Palm kernel cake	10.00
Brewer's dry grain	16.00
Oyster shell	1.75
Salt	1.00
Premix (ruminant)	0.25
Calculated CP	18.0
Calculated metabolizable energy (Kcal/kg)	2400

**Table 3:** Anti-nutritional factors (g/100 g DM) in Guinea grass and water hyacinth

Forage	Anti-nutrients		
	Phenol	Tannin	Saponin
Water hyacinth	2.31	0.86	1.25
Guinea grass	0.08	1.01	0.15

shell and 0.25% fish meal).

Incubation was as reported (Menke and Steingass 1988) using 100 mL calibrated syringes in three batch incubation at 39°C. into 200 mg samples in the syringe was introduced 30 ml inoculums containing cheese cloth rumen liquor and buffer (NaHCO<sub>3</sub> + Na<sub>2</sub>HPO<sub>4</sub> + KCl + 7H<sub>2</sub>O + CaCl<sub>2</sub>·2H<sub>2</sub>O (1:2, v/v). under continuous flushing with carbon dioxide (CO<sub>2</sub>). Gas production rates was recorded at 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60, 72, 84 and 96 hours incubation and each syringe was gently swirled after reading.

At 96 hour of incubation, the content

of each syringe were decanted to determine the ammonia nitrogen and pH of the incubated samples. 4 ml of 10 M NaOH was introduced into the incubated samples as reported (Fievez et al. 2005) to estimate the amount of methane produced at 96 hour incubation. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of the gas produced at intervals was plotted against the incubation time, and from the graph.

Gas production characteristics were estimated using the equation  $Y = a + b(1 - e^{-ct})$  described by Orskov and McDonald (1979),

where: Y = Volume of gas produced at time 't', a = intercept (gas produced from the soluble fraction), c = gas production rate constant for the insoluble fraction (b), t = incubation time. Metabolizable energy ME, (MJ/Kg DM) and organic matter digestibility (OMD %) were estimated as established (Menke and Steingass 1988) and short chain fatty acids (SCFA) was calculated as reported (Getachew et al. 1999).

$$\begin{aligned} \text{ME} &= 2.20 + 0.136 \text{ GV} + 0.057 \text{ CP} + 0.0029 \text{ CF} \\ \text{OMD} &= 14.88 + 0.889 \text{ GV} + 0.45 \text{ CP} + 0.651 \text{ XA} \\ \text{SCFA} &= 0.0239 \text{ GV} - 0.0601 \end{aligned}$$

Where GV, CP, CF and XA are net gas production (ml/200 mg DM), crude protein, crude fibre and ash of incubated samples respectively.

#### Determination of pH and ammonia nitrogen

The supernatant was decanted into a container and pH meter was used to detect the pH. Some of the supernatant were decanted into labeled bottles and stored in the freezer until needed. The ammonia nitrogen was determined according to AOAC (1995).

#### Statistical analysis

Data were subjected to analysis of variance procedure of SAS (1999). Means were separated using Duncan multiple range test of the same software.

## Results

The proximate composition of water hyacinth harvested during the four sub-seasons of the year and that of the grass are shown in Table 1. Dry matter increased from early rain to late dry season, but was generally very low. As the season progressed, recorded crude protein value was similar ( $p > 0.05$ ). Same trend was observed for ether extract and ash content. It was observed that the ash content of the plant was high. The secondary metabolites of water hyacinth and Guinea grass are presented in Table 3. All sampled water hyacinth and Guinea grass contained saponin, tannin and phenol

In vitro gas production characteristics of feedstuff and concentrate are presented in

Table 4. Potential gas production (a+b), gas production from insoluble fraction (b), volume of gas produced (y) and rate of degradation (c). In vitro gas production characteristics varied significantly ( $p < 0.05$ ) among the fermented feedstuffs at this incubation hr. There was apparent decrease in the values of the parameters with an increasing amount of water hyacinth. More and less gas was produced at 0 % and 90 % level of water hyacinth respectively. It was observed that gas production became constant from 72 – 96 hr for diets 2, 3, 4 and 5. Methane (CH<sub>4</sub>), metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) of water hyacinth with guinea grass and concentrate mixtures are shown in Table 5. The amount of CH<sub>4</sub>, ME, OMD and SCFA varied significantly ( $p < 0.05$ ) among the treatment means. It was observed that methane production reduced with reduced inclusion of Guinea grass. There was also apparent decrease in the level of ME, OMD and SCFA as water hyacinth inclusion increased in the mixture.

Figure 1 revealed net gas production of experimental diets. It was observed that diet 1 (0 WH + 90GG + 10CC) produced highest volume of gas compared with other diets throughout the incubation period. The net volume of gas produced at 96 h incubation period also increased significantly ( $p < 0.05$ ) from 56.67 in diet 5 to 82.33 ml/200 mg DM in diet 1. It was observed that gas production for diets 2, 3, 4 and 5 became constant from 72 to 96 hr incubation period.

The Ammonia nitrogen and pH values of experimental diets are shown in Figures 2 and 3, respectively. Effect of treatments on pH of experimental diets was not significant ( $p > 0.05$ ) throughout the incubation period. It was observed that the pH values of the digesta increased progressively among treatments from 6.58 in 24 to 6.9 in 96 hr incubation period. Like the pH level, the Ammonia nitrogen produced from 24 to 96 hr incubation period was not significantly ( $p > 0.05$ ) different among the treatments. It ranged from 5.60 to 15.13 N/100 ml.

**Table 4:** In vitro gas production characteristics of experimental diets at 96 hr incubation

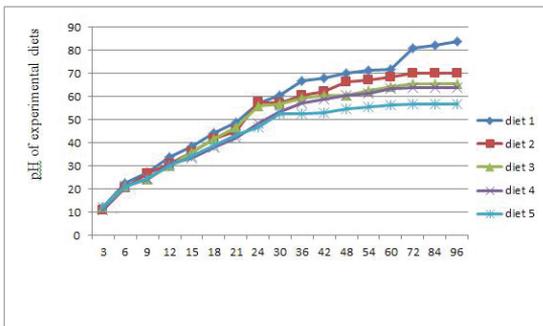
Treatments	b(ml)	a+b (ml)	C(hr)	y (ml)
0WH + 90GG + 10CC	70.33 <sup>a</sup>	82.33 <sup>a</sup>	0.05	84.00 <sup>a</sup>
30WH + 60GG + 10CC	68.67 <sup>a</sup>	74.67 <sup>a</sup>	0.05	70.33 <sup>a</sup>
45WH + 45GG + 10CC	63.67 <sup>ab</sup>	72.33 <sup>ab</sup>	0.04	65.67 <sup>b</sup>
60WH + 30GG + 10CC	59.00 <sup>b</sup>	71.00 <sup>ab</sup>	0.04	63.70 <sup>c</sup>
90WH + 0GG + 10CC	56.33 <sup>c</sup>	67.33 <sup>c</sup>	0.04	56.67 <sup>d</sup>
SEM	0.83	1.21	0.003	5.39

<sup>a, b, c</sup> = means on the same column with different superscripts are significant ( $p < 0.05$ ); b = gas produced from insoluble but degradable fraction (ml / gDM); a+b = potential degradability, y = effective degradability c = rate of degradation, WH = water hyacinth, GG = Guinea grass, CC= concentrate

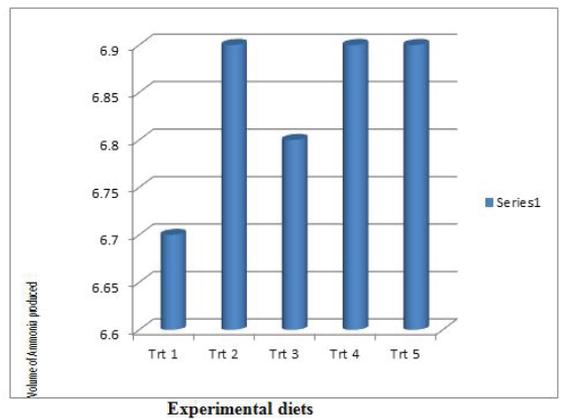
**Table 5:** In vitro gas production parameters of experimental diets at 96 hr incubation Gas production parameters

Parameters	ME (MJ/Kg M)	OMD (%)	SCFA (µmol)	CH <sub>4</sub> (ml)
0WH + 90GG + 10CC	13.68 <sup>a</sup>	98.10 <sup>a</sup>	2.03 <sup>a</sup>	75.0 <sup>a</sup>
30WH + 60GG + 10CC	13.49 <sup>a</sup>	96.98 <sup>a</sup>	2.00 <sup>a</sup>	64.0 <sup>b</sup>
45WH + 45GG + 10CC	13.20 <sup>ab</sup>	94.14 <sup>ab</sup>	1.79 <sup>b</sup>	55.1 <sup>c</sup>
60WH + 30GG + 10CC	13.17 <sup>ab</sup>	92.36 <sup>ab</sup>	1.76 <sup>b</sup>	52.0 <sup>d</sup>
90WH + 0GG + 10CC	12.98 <sup>b</sup>	92.02 <sup>c</sup>	1.72 <sup>b</sup>	50.0 <sup>d</sup>
SEM	0.29	0.27	0.05	0.12

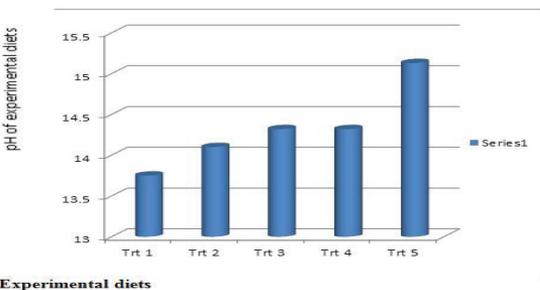
<sup>a, b, c</sup> = means on the same column with different superscripts are significant ( $p < 0.05$ ); b = gas produced from insoluble but degradable fraction (ml / gDM); a+b = potential degradability, y = effective degradability c = rate of degradation, WH = water hyacinth, GG = Guinea grass, CC= concentrate



**Figure 1:** in vitro gas production (ml/200 mg DM) of water hyacinth based diets incubated for 96 hr



**Figure 3:** pH of water hyacinth after 96 hour incubation



**Figure 2:** Ammonia nitrogen of water hyacinth after 96 hour incubation

## Discussion

### *Chemical composition*

The increased dry matter (DM) composition of water hyacinth from early rain to late dry season was a phenomenon which was reported to be due to the effect of age of the forage (Babayemi et al. 2006a). It was observed that the DM was low in all the samples analyzed. Dry matter values ranged from 7.68 (river) to 7.97 (lagoon). The DM obtained for WH in this study is consistent with the report of Mako et al. (2011). This low DM is a limiting factor in feeding the plant on fresh basis to animals since animals will have to take a large quantity to meet requirements. This can be ameliorated by dehydrating the plant under the sun as wilted, hay (Mako and Babayemi, 2008) or as silage (Akinwande et al., 2011). The effect of season on the CP was similar. This agrees with the work of Babayemi et al. (2006a) who reported no seasonal changes in the nutrient composition of water fern as summer progressed in Nigeria. The ash content of the entire plant was high this is a common characteristic of aquatic plants (Nguyen, 1996). The absence of seasonal variation in the ash content of WH suggests a balance of mineral content throughout the year for animals consuming it. The values for NDF fell within the range reported elsewhere for water hyacinth (Aboud et al., 2005). It also compared favorably well with values obtained for some browse plants elsewhere (Ademosun et al., 1988). This value, though high but still fell within the range of NDF of high digestibility (Bamualin 1980).

### *Anti - nutritional factors in water hyacinth and guinea grass*

The two forages (Guinea grass and Water hyacinth) used in this study contained the principal anti nutritional factors found in a number of tropical forages (Fievez et al., 2005). Phenol and saponin contents in WH were higher than those in the Guinea grass, except for tannin. The presence of tannins and saponin in the WH and GG was an added advantage because they have been found to have beneficial effect to ruminants. Saponin is

known to suppress methanogenesis (Babayemi et al. 2004b). Methane is a dietary energy loss and is an important green house gas contributing to global warming (Johnson and Johnson 1995). Tannins had been reported to form complexes with protein in the rumen and remain indigestible due to the high pH and invariably dissociates in the abomasum at a lower pH for proper digestion (Barry and McNabb 1999). The values of saponin and tannin obtained here for the two forages were within the acceptable limit of 5 % for intake and digestibility in ruminants (McLeod 1974). The low content of saponin in the two forages is advantageous, high saponin alone would retard feed intake of ruminants (Onwuka 1990).

### *In vitro gas production and estimated parameters of experimental diets*

Gas production is an indication of degradability of samples (Fievez et al., 2005). The degradation observed in the diets is an indication that water hyacinth (WH) can be used as feed supplements for ruminants. Gas production decreased with increasing inclusion of WH in the diet. This low gas production as level of water hyacinth increased in the diets could be attributed to the presence of saponin, an antinutrient which did not favour the activities of micro-organisms. (Babayemi et al., 2004b), No significant variation occurred from 3 – 24hr period of incubation. Generally the Guinea grass (GG): water hyacinth (WH) combination produced low gas and also high crude protein content of the mixtures because of the inclusion of concentrate. Low gas is produced when substrates are fermented to propionate which is glucogenic, this is beneficial because the energy produced will be available and useful to the animals. The constant production of gas experienced from 72 – 96 for diets with WH inclusion can be attributed also to the presence of anti -nutritional factor in the WH which inhibits the activities of micro organisms (Ajayi et al., 2005).

The utilization of forages is largely dependent on microbial degradation, therefore the rate and potential of gas production would provide a useful basis for the evaluation of

these forages in the diets and potential feed resource. Since gas production is dependent on the relative proportion of soluble and insoluble but degradable and undegradable particles of forage diets, mathematical description of gas production profiles allows evaluation of substrate and fermentability of soluble and slowly fermentable components of feeds (Getachew *et al.*, 1998).

The values of insoluble degradable fraction (b) potential degradability (a + b), rate of degradation (c) and volume of gas produced among WH based diets for 24 and 60hr incubation periods were similar. Implying that any of these diets can be used depending on the availability. However at 96 h incubation period, the values of a rate of degradation (c) was not significant while those of 'b', 'a + b' and 'y' decreased significantly with increasing amount of WH in the diet this might be due to the high fibre content of the forage, which corroborates the work of Nsahlai *et al.* (1994).

The values of 'b', 'a + b' and 'y' in the diet with 30 % WH inclusion were similar to those of the control diet with 0% WH inclusion, but significantly higher than those of 45 %, 60 % and 90 % level of WH inclusion. The implication of this is that 30 % WH inclusion in diets of ruminants will enhance optimal degradability.

The values of 'b' obtained here (56.33 – 70.33) were higher than those reported for dry matter degradation of some tropical legumes and grasses (Ajayi *et al.*, 2007) and also higher than values of 9.5–32.0 ml/200 mg DM reported for some crop residues (Babayemi *et al.*, 2009).

The values of a + b obtained here (67.33 to 82.33 to ml/200 mg DM) compare favorably well with the values reported for dry matter degradation of some legumes and grasses (Ajayi *et al.* 2007), but higher than values reported elsewhere (Babayemi *et al.*, 2009). Although gas production is a nutritionally wasteful product (Mauricco *et al.*, 1999) but provides a useful basis from which Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and SCFA may be predicted. The values of ME (MJ/Kg DM), OMD (%) and SCFA ( $\mu$ mol) of experimental diets differed significantly at 96 hr incubation. The

ME, OMD and SCFA of WH based diets at 96 hr were significantly influenced by the amount of WH included in the diet. However the values obtained here for all these parameters were higher than values reported by Babayemi (2007) in browse plants. Higher values in the present study were expected as concentrates was sparingly included in the diets (Odenyo *et al.*, 1999). The high ME, OMD and SCFA in diet with 30 % inclusion of WH over other diets makes it a better option as supplement feed for ruminants.

Combination of WH: GG in the diets decreased methane Production. The drop in CH<sub>4</sub> when WH:GG were combined other than grass alone showed the presence of a property in WH that may be capable of reducing CH<sub>4</sub> production. In this present study, Saponin was implicated in WH which might be the reason for the drop in CH<sub>4</sub> of the diets containing WH. Saponin inhibits methanogenesis (Fievez *et al.*, 2005; Babayemi *et al.*, 2006a; 2006b)

#### *pH of experimental diets*

The pH range (6.58 6.90) of the digesta after incubation among various diets is within the range for the normal rumen. Grants and Montes (1992) reported pH of 6.9 for normal rumen. The level of pH in the diet in this study as reflected in the digesta showed that lactic acidosis did not occur. The explanation to this could be that since rapidly degradable carbohydrate (the type found in grains) causes accumulation of lactic acid during fermentation make the ruminal pH to drop, it could then be inferred that WH contained slowly degradable carbohydrate.

Carbohydrate fermentation which took place first produced SCFA (propionate, butyrate and acetate) which is acidic, this reduced the pH, before protein fermentation began, since protein fermentation produces branch chain fatty acid (valeric, isovaleric and isobutyrate) which increases the pH. Reason for the progressive increase in the pH values as incubation period progressed.

#### *Ammonia nitrogen of experimental diets*

It is abundantly clear that NH<sub>3</sub> is highly

important for the efficient synthesis of amino acids and therefore microbial protein (Preston and Leng, 1987). The level of NH<sub>3</sub> depends on the type of feed. If highly degradable protein is fed, it will lead to high NH<sub>3</sub> production in the rumen. The level range of NH<sub>3</sub> obtained here (5.6 to 15.13 mg N/100 ml) is higher than the value range of 5 – 8 mg N/100 ml reported by Satter and Slyter (1974) for maximum microbial synthesis. However, these values compared favorably well with the value of 14 mg N/100 ml reported by Schaefer et al. (1980) to achieve 95 % of maximal microbial growth using pure culture.

A downward trend observed in the amount of gas produced with increasing amount of water hyacinth indicated that the forage contained an antinutrient property, while Guinea grass is high in crude fibre and this reduced its digestibility. Digestibility is synonymous to in vitro gas production.

### Conclusion

From the present study it was observed that water hyacinth/ Guinea grass mixture consistently reduced the production of methane. Methane production represents a significant energy loss to ruminants and also contributes to global warming. Since water hyacinth and Guinea grass are two extremes as diets for ruminants, they could be strategically used for optimum performance of ruminants in the tropics. Also, 30 % inclusion of water hyacinth in the diets of ruminants will enhance optimal degradability.

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## BLOOD PROFILE OF EWES DURING THIRD TRIMESTER OF PREGNANCY AND LACTATION

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### Abstract

The study was designed to determine the blood profile of ewes during third trimester of pregnancy and lactation and to establish differences (if any) between the two reproductive states. Twenty-six apparently healthy ewes aged  $\geq 2\frac{1}{2}$  -  $\leq 5$  years and managed intensively were selected from the sheep flock of a farm. The ewes were randomly selected from the pregnant ewes in their third trimester and those lactating. The animals fed on wheat bran, beans offal and base hay with water and salt lick ad libitum. They were assigned into 2 groups comprising of 13 ewes each based on their reproductive status. Ewes in group A were pregnant in third trimester, while ewes in group B were lactating with lambs 1-2 months old. Blood samples were collected before feeding from each group and used for haematological and biochemical analysis. The packed cell volume, haemoglobin, red blood cell and leukocyte count of ewes in group A were not significantly ( $p > 0.05$ ) different from those in group B. However, there were significant ( $p < 0.05$ ) differences in the potassium, bicarbonate and urea concentrations between ewes in groups A and B. The glucose, sodium and creatinine concentrations of ewes in group A were not significantly ( $p > 0.05$ ) different from those in group B. The study has established reference haematological and some selected biochemical values for ewes during third trimester of pregnancy and lactation. This study also established significant differences in the biochemical parameters of potassium, bicarbonate and urea in ewes pregnant in third trimester and lactation.

**Keyword:** Biochemistry, Ewe, Haematology, Lactation, Pregnancy.

## PROFIL SANGUIN DES BREBIS PENDANT LE TROISIÈME TRIMESTRE DE LA GESTATION ET LA LACTATION

### Résumé

L'étude a été conçue pour déterminer le profil sanguin des brebis pendant le troisième trimestre de gestation et la lactation et identifier les différences (le cas échéant) entre les deux états reproductifs. Vingt-six brebis apparemment en bonne santé, âgées de  $\geq 2\frac{1}{2}$  -  $\leq 5$  ans, et élevées en système intensif, ont été sélectionnées dans le troupeau de moutons d'une ferme. Le choix a été fait de manière aléatoire parmi les brebis gestantes ayant atteint le troisième trimestre de gestation et les brebis allaitantes. Les animaux ont été nourris avec du son de blé, des abats aux haricots et du foin de base, l'eau et le sel à lécher étant servis ad libitum. Ils ont été répartis en 2 groupes composés de 13 brebis chacun en fonction de leur état reproductif. Les brebis du groupe A étaient en gestation au troisième trimestre, tandis que les brebis du groupe B allaitaient des agneaux de 1 à 2 mois. Des échantillons de sang ont été prélevés avant le début de l'alimentation de chaque groupe et utilisés pour l'analyse hématologique et biochimique. L'hématocrite, l'hémoglobine, les numérations érythrocytaire et leucocytaire des brebis du Groupe A n'étaient pas significativement ( $p > 0,05$ ) différents de ceux du groupe B. Cependant, des différences significatives ( $p < 0,05$ ) ont été notées au niveau des taux de potassium, de bicarbonate et d'urée entre les brebis des Groupes A et B. Les taux de glucose, de sodium et de créatinine des brebis du Groupe A n'étaient pas significativement différents ( $p > 0,05$ ) de ceux du Groupe B. L'étude a établi des valeurs hématologiques de référence et certaines valeurs biochimiques de référence pour les brebis qui étaient dans le troisième

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trimestre de gestation et les brebis allaitantes. Cette étude a également établi des différences significatives au niveau des paramètres biochimiques du potassium, du bicarbonate et de l'urée chez les brebis gestantes au troisième trimestre et les brebis allaitantes.

**Mots-clés :** biochimie, brebis, hématologie, lactation, gestation.

## Introduction

Blood and their constituents are important and dependable medium for evaluating physiological changes in the physical and health status of an animal (Egbe-Nwiyi *et al.*, 2000; Žvorc *et al.*, 2006; Njidda *et al.*, 2014). This evaluation is accomplished by assessing the haematological and biochemical parameters of the animal, which are affected by age, genetic make-up, sex, housing, transport, environment, nutrition, stress and reproductive status (Egbe-Nwiyi *et al.*, 2000; Balikci *et al.*, 2007). Pregnancy is a reproductive status that extends from fertilization to parturition (Noakes *et al.*, 2001), and causes major physiological, hormonal and biochemical changes in the dam. On the other hand, lactation is the secretion of milk from the mammary gland and begins from the last few days of pregnancy. These physiological states of reproduction induce metabolic changes characterized by alteration in the physiological status of the animal leading to modification of its metabolic activities (Iriadam, 2007; Piccione *et al.*, 2009).

Pregnancy is a reproductive state associated with changes in blood and its constituents. Third trimester is the most stressful period of pregnancy due to increased demand for energy in the fast-growing foetus. Ewes should be in good health condition during and after pregnancy so as to produce viable lambs, failure of this leads to metabolic disturbances such as pregnancy toxemia and milk fever (hypocalcaemia) characterized by anorexia, blindness, depression, staggering gait, recumbency, weakness, coma and death in severe cases leading to economic losses. In addition, ewes that recover from diseases during pregnancy may have difficulty in parturition, die during the process or develop retained placenta and metritis. Reference haematological and biochemical values for

ewes during the third trimester of pregnant and lactation is lacking in our tropical environment. This has led to wrong assumptions on the actual health status of the ewe particularly when dystocia occurs and surgical intervention is required. Although information on the haematological and biochemical profile of indigenous sheep has been reported (Saror and Van Veen, 1979), there is paucity of information on these profiles in the ewes during their third trimester of pregnant and lactation to the best of our knowledge. The information will assist in validating history provided and physical examination, thereby aiding intervention particularly during metabolic diseases and dystocia. This study is therefore aimed at determine the haematological and biochemical profile of ewes during third trimester of pregnancy and lactation.

## Materials and Methods

### Study Location

The study was carried out in Sokoto, Nigeria. Sokoto lies between 130 and 140N and 50 and 60E. It has an average annual temperature of 28.30C and an annual rainfall of about 500 mm with the highest peak in August (Uluocha and Ekop, 2000).

### Experimental Animals

Twenty-six apparently healthy ewes aged  $\geq 2\frac{1}{2}$  -  $\leq 5$  years comprising Uda, Balami, Sudanese and Balami/Sudanese cross were used in this study. Their ages were obtained from the farm's records but correlated with observations made on the pattern of their dental eruption as described by Wosu (2002). The ewes are part of the sheep flock of a livestock farm, located in Sokoto metropolis. The sheep flock is made up of rams, ewes and lambs numbering 155 and intensively managed in pens. Natural mating is practiced on the farm.

Veterinary services of routine deworming and treatment of clinically sick animals is carried out by the Veterinary Teaching Hospital, Usmanu Danfodiyo University, Sokoto. The animals used were randomly selected from the apparently healthy pregnant ewes in their third trimester and those lactating for 1-2 months. They fed on wheat bran, beans offal and base hay with water and salt lick ad libitum.

#### *Experimental Design*

They ewes were assigned based on their reproductive status into two groups comprising 13 ewes each. Ewes in group A were pregnant in third trimester, while ewes in group B were lactating with lambs 1-2 months old. The trimester of pregnancy was determined using abdominal ballottement and history provided by their handlers. In addition, the day of sample collection was noted and its difference with the day of parturition was determined to ensure the ewes were in third trimester. Ewes with differences more than 50 days were excluded from the study. Their lactating status was determined based on the history of lambing and presence of suckling lamb.

#### *Blood Collection*

Seven millilitres of blood was collected from each ewe in the morning before feeding via venipuncture. Out of this, 2 ml was dispensed into sample bottle containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant and used for haematological evaluation, while the remaining 5 ml was dispensed into another sample bottle without anticoagulant and used for selected biochemical evaluation.

#### *Haematological Evaluation*

Packed cell volume, haemoglobin, red blood cells, white blood count and differential leukocyte counts were determined using the methods described by Schalm et al. (2010).

#### *Biochemical Evaluation*

Glucose, sodium, potassium, bicarbonates, urea, creatinine and ketone bodies were determined. Glucose concentration

was determined using a glucometer (ACUU-CHEK®). Briefly, a drop of freshly collected blood from the animal was placed on a test strip. The strip absorbs the blood, determines the glucose (mg/dL) concentration and displays the result. The biochemical parameters of sodium, potassium, bicarbonates, urea, creatinine and ketone bodies were analysed following the methods described by Coles (1986).

#### *Data Analyses*

Data obtained were statistically analysed using unpaired Student t-test (GraphPad, 2000) and presented in tables. Values of  $p < 0.05$  were considered statistically significant.

## **Results**

The packed cell volume, haemoglobin and red blood cell value of ewes during third trimester of pregnancy and lactation is presented in Table 1. The mean PCV of pregnant ewes in third trimester and in lactation were 31.46 % and 29.25 %, respectively. These values were not significantly ( $p > 0.05$ ) different from each other. There was no significant ( $p > 0.05$ ) difference between mean haemoglobin of ewes during third trimester of pregnancy (10.05 g/dL) and lactation (9.77 g/dL). The red blood cell of ewes during third trimester of pregnancy (10.80 g/dL) and lactation (10.04) was also not significantly ( $p > 0.05$ ) different from each other.

Total and differential leukocyte counts of pregnant ewes in third trimester and lactation are presented in Table 2. The total leukocyte counts were  $7.98 \times 10^3/L$  and  $7.17 \times 10^3/L$  for ewes during third trimester of pregnancy and lactation, respectively. These were not significantly ( $p > 0.05$ ) different from each other. There were also no significant ( $p > 0.05$ ) differences in the neutrophil, lymphocyte, monocyte, eosinophil and bands of ewes during third trimester of pregnancy and lactation.

The mean of selected biochemical parameters of ewes during third trimester of pregnancy and lactation are presented in Table 3. There were significant ( $p < 0.05$ ) differences between potassium, bicarbonate and urea

**Table 1:** Mean  $\pm$  SD packed cell volume, haemoglobin and red blood values of ewes during third trimester of pregnancy and lactation

Parameter	Reproductive status	
	Pregnant (n=13)	Lactating (n=13)
PCV (%)	31.46 $\pm$ 3.87	29.25 $\pm$ 2.26
Hb (g/dL)	10.05 $\pm$ 1.30	9.77 $\pm$ 0.61
RBC (x 106)	10.80 $\pm$ 1.53	10.04 $\pm$ 0.92

Key: PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red blood cell

**Table 2:** Mean  $\pm$  SD total and differential leukocyte counts of ewes during third trimester of pregnancy and lactation

Parameter	Reproductive status	
	Pregnant (n=13)	Lactating (n=13)
WBC (109/L)	7.98 $\pm$ 1.24	7.17 $\pm$ 1.45
Neu (109/L)	4.07 $\pm$ 1.05	4.07 $\pm$ 1.18
Lym (109/L)	3.58 $\pm$ 1.25	2.73 $\pm$ 0.91
Mono (109/L)	0.11 $\pm$ 0.10	0.17 $\pm$ 0.12
Eosin (109/L)	0.20 $\pm$ 0.17	0.20 $\pm$ 0.13
Bands (109/L)	0.02 $\pm$ 0.05	0.01 $\pm$ 0.02

Key: WBC: White blood cell; Neu: Neutrophil; Lym: Lymphocyte; Mono: Monocyte; Eosin: Eosinophil; Baso: Basophil

**Table 2:** Mean  $\pm$  SD total and differential leukocyte counts of ewes during third trimester of pregnancy and lactation

Parameter	Reproductive status	
	Pregnant (n=13)	Lactating (n=13)
Glucose (mg/dL)	48.23 $\pm$ 13.72	55.77 $\pm$ 6.60
Ketone bodies	0/13	1/13
Sodium (Mmol/L)	140.54 $\pm$ 3.46	139.92 $\pm$ 4.44
Potassium (Mmol/L)	4.46 $\pm$ 0.22 <sup>aa</sup>	4.22 $\pm$ 0.20 <sup>ab</sup>
Bicarbonate (Mmol/L)	22.31 $\pm$ 1.25 <sup>aa</sup>	21.23 $\pm$ 0.73 <sup>ab</sup>
Urea (Mmol/L)	51.12 $\pm$ 9.70 <sup>aa</sup>	60.25 $\pm$ 7.14 <sup>ab</sup>
Creatinine (Mmol/L)	0.75 $\pm$ 0.17	0.75 $\pm$ 0.17

values within the same row with different superscripts are statistically significant ( $p < 0.05$ )

concentrations of ewes during third trimester of pregnancy and those of lactation. However, there were no significant ( $p > 0.05$ ) differences in the glucose, sodium and creatinine concentrations of ewes during third trimester of pregnancy and those of lactation (Table 3). All the pregnant ewes were negative for ketone bodies, while only one of the lactating ewes was positive for ketone bodies.

## Discussion

The packed cell volume (PCV), red blood cell (RBC) and haemoglobin (Hb) of pregnant ewes in third trimester were insignificantly higher than those in lactating ewes. These values are similar to the values reported by Saror and Van Veen (1977) in apparently healthy sheep in the Guinea Savanna region of Nigeria, as well as Antunović et al. (2011) in Croatia. The insignificant difference in PCV, RBC and Hb between the two physiological

states is also similar to the report of Ate et al. (2009b) in cows, but contradicts the report of Žvorc et al. (2006) in pigs probably due to the specie variation. Parturition in sheep has been reported to be associated with increase in PCV, RBC and Hb that returns to pre partum values fourteen days postpartum (Anosa and Ogbogu, 1979). Samples for the lactating ewes were collected 4-8 weeks post-partum thereby suggesting the return of the values to their pre partum levels.

Blood leukocyte counts of ewes in third trimester of pregnancy and lactation were not significantly different. This is similar to the report of Ate et al. (2009b) in pregnant and lactating cows as well as the report of Žvorc et al. (2006) in pregnant sows. However, the values obtained in this study are lower than the reports of Saror and Van Veen (1977) in non-pregnant sheep. The stress of pregnancy and lactation may be responsible for this. These reproductive states are associated with hormonal changes particularly steroid hormones which are known to be immunosuppressive (Wyle and Kent, 1977). Progesterone and cortisol levels are elevated during pregnancy (Hoehn and Marieb, 2010) and lactation (Arthur and Green, 1983), and these hormones are potent immune-suppressants (Szekeres-Bartho, 1992). There were no significant differences in the neutrophils, lymphocyte, monocytes, eosinophils and band cells of ewes in third trimester of pregnancy and lactation. This is contrary to the report of Ate et al. (2009b), who observed significant differences in cows. The differences may probably be due to the varying periods of sampling in the two studies. Ate et al. (2009b) sampled lactating cows within 2 weeks of parturition, unlike the 4 – 8 weeks post-partum ewes used in this study. Anosa and Ogbogu (1979) reported leukocytosis characterized by neutrophilia, lymphocytopenia and eosinopenia in ewes at parturition. However, the values returned to pre-partum values within 2 weeks of parturition. This may account for the insignificant differences observed in this study. In comparison with apparently healthy sheep sampled by Saror and Van Veen (1977), a leukopenia characterized

with lymphocytopenia, eosinopenia and monocytopenia is suggestive during the third trimester of pregnancy and lactation.

The mean blood glucose concentration of pregnant ewes in third trimester is similar to the report of El-Sherif and Assad (2001) in Barki ewes. Their concentration is lower than the mean concentration in lactating ewes. However, it was not statistically significant. The insulin responsiveness of ewes in third trimester of pregnancy is usually reduced, leading to decrease in uptake by tissues (Sporleder, 1998). Apart from this, late pregnancy is generally associated with increased demand for glucose due to rapidly developing foetus (Firat and Özpınar, 2002; Kulcsár et al., 2006). None of the ewes in third trimester of pregnancy and lactation had ketonaemia except one lactating ewe which is an isolated case. However, healthy animals have small quantities of ketone bodies but large quantities are present during starvation and exercise (Koeslag et al., 1980). Glucose is an indicator in starvation and there was no significant variation in the glucose concentration of ewes in the two physiological states (pregnancy and lactation).

There were no significant differences between the sodium concentration of ewes in third trimester of pregnancy and lactation. This is similar to the report of Ate et al. (2009a) in cattle, but contradicts the report of Žvorc et al. (2006) in sow, who observed lower sodium concentration in lactating than pregnant sows. This is probably due to the large litter size of sow and variation in specie. Pregnancy and lactation are associated with high demand for water by the foetus and milk secreting udder leading to hyponatremia (Olsson et al., 1996.). Low blood sodium is caused by adrenal insufficiency (Liamis et al., 2011) due to alterations in renal physiology during pregnancy and lactation (Arthur and Green, 1983; Cheung and Lafayette, 2013). Potassium concentration was significantly higher in pregnant ewes than in lactating ewes. Žvorc et al. (2006) also observed higher potassium concentration during the pre-partum period than post-partum in sow, although not statistically significant. However, Ate et al. (2009a) reported an insignificant

difference in potassium levels in cows during third trimester of pregnancy and lactation. Potassium is believed to increase during late pregnancy and decrease after parturition (Belyea *et al.*, 1975); this may have caused the significant difference. There was significant increase in bicarbonate concentration of pregnant ewes in third trimester than lactating ewes. This is consistent with the report of Ate *et al.* (2009a) in cows. The high level of progesterone during pregnancy may be responsible for this. Progesterone causes hyperventilation leading to increase carbon dioxide hydration in blood (Bayliss *et al.*, 1987).

Blood urea levels of pregnant ewes in third trimester were significantly lower than those of lactating ewes. This is consistent with the observations of Antunović *et al.* (2011) in Tsigai ewes but contradicts the report of Ndibualonji *et al.* (1998) and Piccione *et al.* (2009), who observed an insignificant increase in third trimester of pregnancy over lactating Corriedale and Comisana ewes, respectively. Breed and nutrition may be responsible for this variation. Urea levels are known to decrease during pregnancy, due to increase in glomerular filtration rate that peaks at parturition (Cheung and Lafayette, 2013). In addition, the greater demand of lactation than pregnancy on the dam (Arthur and Green, 1983) could be another factor. It is also possible that the ewes used by Ndibualonji *et al.* (1998) and Piccione *et al.* (2009) were on high protein diet. Urea is the end product of protein metabolism (Harper, *et al.*, 2003), therefore high protein diet would lead to high urea production. Creatinine level of pregnant ewes in third trimester was not significantly different from lactating ewes. This is consistent with the report of Piccione *et al.* (2009) in Comisana sheep but inconsistent with the report of El-Sherif and Assad (2001) in Barki sheep probably due to breed variation. However, increased progesterone and prolactin levels during pregnancy and lactation have been implicated in renal dysfunction (Arthur and Green, 1983; Cheung and Lafayette, 2013) and this leads to increased blood creatinine levels (Coresh *et al.*, 2001). Piccione *et al.* (2009) also observed significantly higher creatinine levels in

dry ewes than in third trimester ewes, which were attributed to increased rate of muscle synthesis to replenish body reserves.

In conclusion, the study has established reference haematological values for ewes during third trimester of pregnancy and lactation, respectively. It has also established reference values for glucose, sodium, potassium, bicarbonate, urea and creatinine. In addition, the study revealed significant differences in the potassium, bicarbonate and urea concentrations of ewes during third trimester of pregnancy and lactation.

Based on this study, it is recommended that the study be extended to include other biochemical parameters with a view of establishing their reference values. Similar studies may be carried out to cover other reproductive states of the ewe.

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### Impact of research findings

The packed cell volume, haemoglobin, red blood cell and leukocyte values of pregnant ewes in third trimester are not significantly different from those of lactating ewes. There were significant differences in values for some selected biochemical parameters (potassium, bicarbonate and urea). The glucose, ketone bodies, sodium and creatinine concentrations of ewes during third trimester of pregnancy were comparable with those of lactating ewes.

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## ORGAN BACTERIOLOGICAL DYNAMICS IN *Heterobranchus bidorsalis* JUVENILES FED DIETS FORTIFIED WITH *Lactobacillus fermentum*, *Saccharomyces cerevisiae* AND THEIR COMBINATION

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### Abstract

The effect of dietary intake of *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and their combination on selected tissue bacteriology of *Heterobranchus bidorsalis* juveniles was investigated. Ten experimental diets at 41.0% crude protein were prepared to include Lf1 (basal diet + 10<sup>1</sup>cfu/mL *Lactobacillus fermentum*), Lf2 (basal diet + 10<sup>2</sup>cfu/mL *Lactobacillus fermentum*), Lf3 (basal diet + 10<sup>3</sup>cfu/mL *Lactobacillus fermentum*), Sc1 (basal diet + 10<sup>1</sup>cfu/mL *Saccharomyces cerevisiae*), Sc2 (basal diet + 10<sup>2</sup>cfu/mL *Saccharomyces cerevisiae*), Sc3 (basal diet + 10<sup>3</sup>cfu/mL *Saccharomyces cerevisiae*), LfSc1 (basal diet + 10<sup>1</sup>cfu/mL *Lactobacillus fermentum* and 10<sup>1</sup>cfu/mL *Saccharomyces cerevisiae*), LfSc2 (basal diet + 10<sup>2</sup>cfu/mL *Lactobacillus fermentum* and 10<sup>2</sup>cfu/mL *Saccharomyces cerevisiae*), LfSc3 (basal diet + 10<sup>3</sup>cfu/mL *Lactobacillus fermentum* and 10<sup>3</sup>cfu/mL *Saccharomyces cerevisiae*) and control (basal diet).

The experiment was replicated thrice with the fish (initial mean weight 22.76±0.83g) fed twice daily at 3% body weight for twelve weeks. Microbial analyses of experimental water, fish organs and organ index of the experimental fish were investigated. Data were statistically analyzed using descriptive statistics and one-way analysis of variance (ANOVA) at  $p \leq 0.05$ .

Results of enterobacteria counts and total viable counts from this study showed that bacterial loads in water and experimental fish were significantly affected by *L. fermentum* and *S. cerevisiae* than the control group at 4th, 8th and 12th weeks. Furthermore, higher values for the skin (somatic index) than other organs were observed, since it is the area of first contact, which received most of the microorganisms than other organs in all the treatments. The result of organ indices further revealed that the spleen, liver, gill, intestine and kidney were insignificantly increased in both the treated and the control groups. Therefore, *Lactobacillus fermentum* and *Saccharomyces cerevisiae* inclusion in the diet of *Heterobranchus bidorsalis* could be a promising dietary additive which could positively influence growth, reduce and prevent any bacterial infection in fish culture.

**Keywords:** *Lactobacillus fermentum*, *Saccharomyces cerevisiae*, *Heterobranchus bidorsalis*, Enterobacteriaceae.

## DYNAMIQUES BACTERIOLOGIQUES DES ORGANES DES JUVENILES *Heterobranchus bidorsalis* NOURRIS AUX REGIMES ENRICHIS AVEC *Lactobacillus fermentum*, *Saccharomyces cerevisiae* ET LEUR COMBINAISON

### Resume

La présente étude a examiné l'effet de l'absorption de *Lactobacillus fermentum*, *Saccharomyces cerevisiae* et de leur combinaison sur la bactériologie de certains tissus de juvéniles *Heterobranchus bidorsalis*. Dix régimes expérimentaux à 41.0% de protéine brute ont été préparés de la manière suivante : Lf1 (régime de base + 10<sup>1</sup>cfu/mL *Lactobacillus fermentum*) ; Lf2 (régime de base + 10<sup>2</sup>cfu/mL *Lactobacillus fermentum*) ; Lf3 (régime de base + 10<sup>3</sup>cfu/mL *Lactobacillus fermentum*) ; Sc1 (régime de base + 10<sup>1</sup>cfu/mL *Saccharomyces cerevisiae*) ; Sc2 (régime de base + 10<sup>2</sup>cfu/mL *Saccharomyces cerevisiae*) ; Sc3 (régime de

base +  $10^3$ cfu/mL *Saccharomyces cerevisiae*) ; LfSc1 (régime de base +  $10^1$ cfu/mL *Lactobacillus fermentum* et  $10^1$ cfu/mL *Saccharomyces cerevisiae*) ; LfSc2 (régime de base +  $10^2$ cfu/mL *Lactobacillus fermentum* et  $10^2$ cfu/mL *Saccharomyces cerevisiae*) ; LfSc3 (régime de base +  $10^3$ cfu/mL *Lactobacillus fermentum* et  $10^3$ cfu/mL *Saccharomyces cerevisiae*) ; et le régime témoin (régime de base).

L'expérience a été répétée trois fois, les poissons (poids moyen initial  $22,76 \pm 0,83$  g) étant nourris deux fois par jour à 3% de poids corporel pendant douze semaines. Des analyses microbiennes de l'eau expérimentale, des organes de poissons et de l'indice d'organes des poissons expérimentaux ont été étudiées. Les données ont été analysées statistiquement à l'aide de statistiques descriptives et de l'analyse de variance unidirectionnelle (ANOVA) à  $p < 0,05$ .

Les résultats des numérations d'entérobactéries et des numérations totales viables de cette étude ont montré que les charges bactériennes dans l'eau et les poissons expérimentaux ont été significativement affectées par *L. fermentum* et *S. cerevisiae* par rapport au groupe témoin aux 4ème, 8ème et 12ème semaines. En outre, des valeurs plus élevées pour la peau (indice somatique) par rapport aux autres organes ont été observées, car la peau est la zone de premier contact qui a reçu la plupart des microorganismes en comparaison avec les autres organes dans tous les traitements. Le résultat des indices d'organes a révélé en outre que la rate, le foie, la branchie, l'intestin et le rein avaient augmenté de volume mais de manière non significative dans les groupes traités et le groupe témoin. Par conséquent, l'inclusion de *Lactobacillus fermentum* et de *Saccharomyces cerevisiae* dans l'alimentation des *Heterobranchus bidorsalis* pourrait être un additif alimentaire prometteur susceptible d'influencer positivement la croissance, de réduire et de prévenir toute infection bactérienne dans la culture des poissons.

**Mots-clés:** *Lactobacillus fermentum*, *Saccharomyces cerevisiae*, *Heterobranchus bidorsalis*, Enterobacteriaceae.

## Introduction

Production of quality fish which is able to grow optimally is the priority of aquaculture business. However, the disease outbreak in fish farming has been reported to be a major obstacle worldwide thus, causing economic loss to the industry (Bello *et al.*, 2012). Efforts are therefore, being engaged towards reducing the fish loss which could be as the result of stress and disease encountered in water, which could eventually affect the economic performance of the aquaculture business. Narvaez *et al.* (2010) described parts of fish such as skin, gills, or gastrointestinal tract as the areas of first contact by potential pathogens, therefore make them vulnerable to microbial infections.

Prophylaxis and antibiotic treatments in aquaculture have been reported to lead to microorganisms being resistant to antibiotics, thus leading to retention of antibiotics residues in fish flesh and aquatic environment (Lara-Flores, 2011). Therefore, to alleviate the potential threat of diseases to human and animal health, an eco-friendly and preventive measures such as the use of microorganisms as probiotics in disease management should be

adopted (Lara-Flores, *et al.*, 2010).

Anderson, (1992) reported immunostimulants to improve the non-specific defense mechanism, increase the resistance to diseases and infection, leading to growth improvement in fish. Nevertheless, attention is being given to immunostimulants and several immunostimulants have been found to be effective in different fish species (Cerezuela *et al.*, 2009). Biological production of fish is fast gaining acceptance over the last decade, consequently giving natural immunostimulants more consideration. Experimental fish fed natural immunostimulant was reported to have its immune system improved (Saad *et al.*, 2013).

*Lactobacillus fermentum* and *Saccharomyces cerevisiae* isolated from fermented food, corn slurry 'ogi' and palm wine respectively could be considered as immunostimulants in fish production due to their high antimicrobial and antibacterial properties (Zapata, 2013). The study examined the likely effect of *L. fermentum* and *S. cerevisiae* as potential antimicrobials in the farming of *H. bidorsalis*.

**Materials and Methods**

*Isolation Procedure*

*Isolation of Lactobacillus fermentum and Saccharomyces cerevisiae*

Lactobacillus fermentum was isolated from corn slurry ‘Ogi’ according to Ogunshe and Olabode (2009), while Saccharomyces cerevisiae was isolated from palm wine according to Ukwuru and Awah (2013).

*Media preparation*

All media used were prepared according to manufacturer’s instruction as described by Akanmu et al., (2016).

*Experimental diets formulation*

Proximate compositions of the diets used for this study are as follows: 41.0 % crude protein, 13.81 % crude fiber, 16.34 % Ash, 18.34 % ether extract and 10.4 % moisture. Ten (10) experimental diets were formulated viz a viz: Lf1 (basal diet + 10<sup>1</sup>cfu/mL of L. fermentum), Lf2 (basal diet + 10<sup>2</sup>cfu/mL of L. fermentum), Lf3 (basal diet + 10<sup>3</sup>cfu/mL of L. fermentum), Sc1 (basal diet + 10<sup>1</sup>cfu/mL of S. cerevisiae), Sc2 (basal diet + 10<sup>2</sup>cfu/mL of S. cerevisiae), Sc3

(basal diet + 10<sup>3</sup>cfu/mL of S. cerevisiae), LfSc1 (basal diet + 10<sup>1</sup>cfu/mL of L. fermentum and 10<sup>1</sup>cfu/mL of S. cerevisiae) LfSc2 (basal diet + 10<sup>2</sup>cfu/mL of L. fermentum and 10<sup>2</sup>cfu/mL of S. cerevisiae) LfSc3 (basal diet + 10<sup>3</sup>cfu/mL of L. fermentum and 10<sup>3</sup>cfu/mL of S. cerevisiae) and control (basal diet). Feed ingredients such as fishmeal (72%), soya bean meal, groundnut cake, maize, lysine, starch, vegetable oil, di-calcium phosphate, fish mineral premix, salt, methionine, and vitamin C were mixed thoroughly with water and cultured isolates. The resulting dough was made into pellets, dried with sun and stored in airtight enclosures, which were kept at room temperature to prevent the growth of mold on the feed.

*Microbiological analysis of experimental water*

Samples of experimental water were collected every 4th week in sterile glass beakers. To 1 mL of water sample was added 9 mL of distilled water to make a stock solution of 10<sup>1</sup>cfu/mL which was serially diluted to 10<sup>3</sup>cfu/mL. One (1) mL of each diluent was dispensed into two petri dishes such that one petri dish contained plate count agar used for total bacterial count determination following

**Table 1:** Microbial load in experimental water used for rearing *Heterobranchus bidorsalis* juveniles fed diets fortified observed during the experiment

TRT	4TH WEEK		8TH WEEK		12TH WEEK	
	Enterobact eria Counts (10 <sup>1</sup> cfu/ mL)	Total Viable Counts (10 <sup>1</sup> cfu/ mL)	Enterobact eria Counts (10 <sup>2</sup> cfu/ mL)	Total Viable Counts (10 <sup>2</sup> cfu/ mL)	Enterobact eria Counts (10 <sup>3</sup> cfu/ mL)	Total Viable Counts (10 <sup>3</sup> cfu/ mL)
Lf <sup>1</sup>	3.7±0.01 <sup>d</sup>	3.8±0.01 <sup>b</sup>	3.5±0.02□	3.8±0.01□	3.6±0.01□	4.1±0.01□
Lf <sup>2</sup>	3.7±0.01 <sup>d</sup>	4.0±0.02 <sup>d</sup>	3.7±0.01□	4.2±0.01□	3.2±0.01□	4.1±0.01□
Lf <sup>3</sup>	3.6±0.01 <sup>c</sup>	4.0±0.01 <sup>d</sup>	3.7±0.01□	3.9±0.01□	3.0±0.01□	3.5±0.01□
Sc <sup>1</sup>	3.4±0.00 <sup>a</sup>	3.6±0.01 <sup>a</sup>	3.5±0.02□	3.7±0.01□	3.0±0.00□	3.2±0.01□
Sc <sup>2</sup>	3.7±0.01 <sup>d</sup>	3.8±0.01 <sup>b</sup>	3.2±0.01□	4.4±0.01□	3.8±0.01□	4.2±0.01□
Sc <sup>3</sup>	3.5±0.01 <sup>b</sup>	3.8±0.01 <sup>b</sup>	4.3±0.02□	4.9±0.01□	4.6±0.01□	4.9±0.01□
LfSc <sup>1</sup>	3.7±0.01 <sup>d</sup>	3.9±0.02 <sup>c</sup>	3.0±0.01□	3.6±0.01□	3.0±0.01□	3.3±0.02□
LfSc <sup>2</sup>	3.8±0.01 <sup>e</sup>	3.8±0.02 <sup>d</sup>	3.8±0.02□	4.3±0.02□	3.2±0.01□	4.5±0.01□
LfSc <sup>3</sup>	3.7±0.02 <sup>d</sup>	3.9±0.01 <sup>c</sup>	3.8±0.03□	4.4±0.01□	3.0±0.01□	4.0±0.01□
Ctrl	5.0±0.02 <sup>f</sup>	5.1±0.01 <sup>e</sup>	5.0±0.02□	5.3±0.02□	5.0±0.01□	5.8±0.03□

Means with the same letter along the column are not significantly different (p>0.05)

**Table 1:** Microbial load in experimental water used for rearing *Heterobranchus bidorsalis* juveniles fed diets fortified observed during the experiment

TRTS	Kidney (g)	Skin (g)	Liver (g)	Gill (g)	Spleen (g)	Intestine (g)
Lf <sup>1</sup>	0.60±0.01□	21.4±0.02□	1.05±0.01□	3.40±0.01□	0.03±0.00□	2.75±0.01□
Lf <sup>2</sup>	0.59±0.00□	20.1±0.01□	0.95±0.00□	2.83±0.01□	0.05±0.01□□	3.19±0.01□
Lf <sup>3</sup>	0.74±0.01□	20.8±0.01□	1.21±0.01□	3.37±0.01□	0.05±0.00□□	2.64±0.01□
Sc <sup>1</sup>	0.35±0.00□	19.4±0.00□	0.79±0.01□	2.40±0.01□	0.45±0.02□	3.11±0.00□
Sc <sup>2</sup>	0.54±0.01□	19.9±0.00□	1.10±0.02□	2.83±0.01□	0.05±0.00□□	2.54±0.01□
Sc <sup>3</sup>	0.46±0.01□	21.0±0.02□	0.95±0.02□	2.66±0.01□	0.06±0.01□	2.68±0.00□
LfSc <sup>1</sup>	0.49±0.00□	17.9±0.01□	0.90±0.01□	2.68±0.02□	0.05±0.01□□	2.24±0.02□
LfSc <sup>2</sup>	0.60±0.01□	25.7±0.01□	1.29±0.01□	3.75±0.01□	0.03±0.00□	2.71±0.01□
LfSc <sup>3</sup>	0.60±0.01□	27.4±0.02□	1.39±0.00□	4.19±0.01□	0.05±0.01□□	2.91±0.00□
Ctrl	0.56±0.00□	28.5±0.01□	1.42±0.01□	4.24±0.01□	0.04±0.01□□	2.95±0.01□

pour plate count method (APHA, 1985) and the other petri dish had Mac Conkey agar used for total coliform count according to Hitchins *et al.*, (1995). The petri dishes were gently swirled for a few times and were later incubated for 24 hours at 37°C.

Samples of the fish organs (skin, gill, liver, intestine, spleen and kidney) were collected monthly during the study for microbiological analysis. To 1g sample of fish organ collected was added 9ml of distilled water and crushed in the mortar and pestle. 1mL of the suspension obtained was serially diluted to 10<sup>3</sup>. 1mL from each diluent was emptied into two petri dishes with a petri dish having plate count agar while, the other had Mac Conkey agar. These petri dishes were incubated for 24 hours at 37°C. The colonies were counted using colony counter after the samples of water and organs collected were incubated to determine the total viable and enterobacteriaceae counts of the microbes in the samples. The results were however expressed in cfu/mL.

*Determination of weights of organs of H. Bidorsalis juveniles fed diets fortified with Lactobacillus Fermentum, Saccharomyces cerevisiae and their combination.*

Three fish from each experimental treatment were sacrificed for the harvest of the following organs; skin, gill, kidney, liver, intestine and spleen. The harvested organs

were weighed and the organ indices were determined according to Fox *et al.*, (1997).

#### Statistical analysis

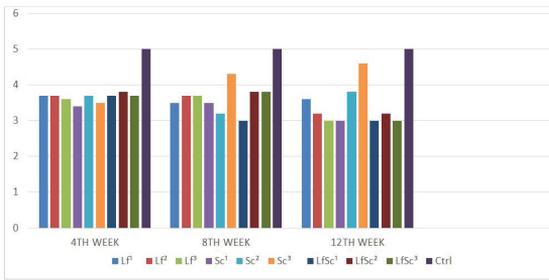
Bacteriological characteristics and organ indices resulting from the experiment were analysed with one-way analysis of variance (ANOVA) using SPSS 2013, Version 21.0. Differences among the individual means were compared using Duncan's Multiple Range Test.

## Results

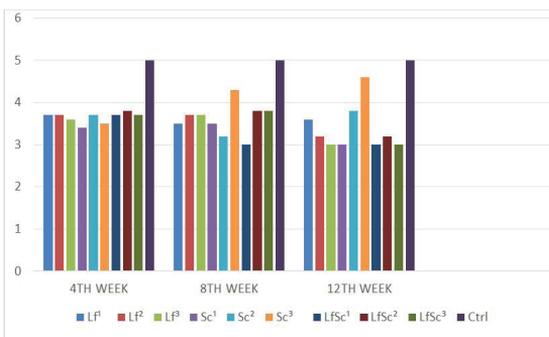
Microbiological analyses of experimental water used in holding *Heterobranchus bidorsalis* juveniles fed diets fortified with *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and their combination.

#### Microbial analysis of experimental water monitored during growth experiment

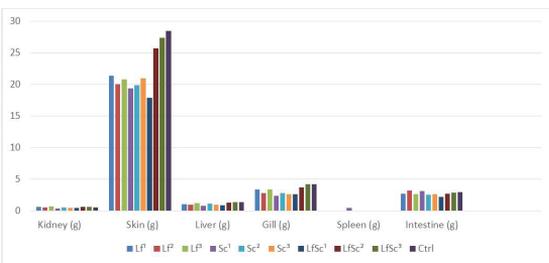
The microbial load in experimental water during the growth study was presented in Table 1 and illustrated in Figures 1 and 2. The enterobacteriaceae count (EBC) in the experimental water ranged from 5.0±0.01 cfu/mL (control) in weeks 4, 8 and 12 to 3.0±0.01 cfu/mL in LfSc1 in week 8, LfSc1 and LfSc3 in week 12. Meanwhile, the total viable counts (TVC) of the microbial in the experimental water during the growth study ranged between 5.8±0.03 cfu/mL (control) in week 12 to 3.2±0.01 cfu/



**Figure 1:** Enterobacteriaceae count of the microbes present in experimental water sample of *H. bidorsalis* fed diets containing *L. fermentum*, *S. cerevisiae* and their combination.



**Figure 2:** Total viable count of the microbes presents in experimental water sample of *H. bidorsalis* fed diets containing *L. fermentum*, *S. cerevisiae* and their combination.



**Figure 3:** Weights of harvested organs from *H. bidorsalis* fed *L. fermentum* and *S. cerevisiae* fortified diets.

mL (Sc1) also in week 12. Highest TVC in 4th, 8th and 12th weeks of the experiment with average values of  $5.1 \pm 0.01$  cfu/mL,  $5.3 \pm 0.02$  cfu/mL, and  $5.8 \pm 0.03$  cfu/mL, respectively were recorded in control. The lowest TVC ( $3.6 \pm 0.01$  cfu/mL) were recorded in the experimental water containing fish fed diets Sc1 and LfSc1 in weeks 4 and 8 respectively, while,  $3.2 \pm 0.01$  cfu/mL was observed in those fed diet Sc1 in 12th

week. The study showed experimental water containing fish fed with diet Sc3, had highest microbial load among the fortified diets in weeks 8 and 12.

*Weights of organ of Heterobranchus bidorsalis juveniles fed diets fortified with Lactobacillus fermentum, Saccharomyces cerevisiae and their combination.*

The weights of the organs harvested from *H. bidorsalis* juveniles fed *L. fermentum*, *S. cerevisiae* and their combination during growth study was presented in Table 2 and illustrated in Figure 3. The weight of kidney ranged from  $0.35 \pm 0.00$  g (Sc1) to  $0.74 \pm 0.00$  g (Lf3). Lower weights of kidney were recorded in fish fed diets containing Sc at 101, 103 and LfSc1 at 101cfu/mL inclusion levels, compared to those fed control diet. Experimental fish fed with other fortified diets recorded higher kidney weights, except the ones fed with Sc2 which had lower kidney weight when compared to those fed control diet. Fish fed control diet recorded significantly ( $P < 0.05$ ) highest skin, liver, and gills weights ( $28.5 \pm 0.01$  g,  $1.42 \pm 0.01$  g and  $4.24 \pm 0.01$  g respectively), while lowest skin weight ( $17.9 \pm 0.01$  g) was recorded in LfSc1, fish fed diet enriched with Sc1 had  $0.79 \pm 0.01$  g and  $2.40 \pm 0.01$  g as the lowest weights for liver and gills, respectively. The weight of spleen varied from  $0.06 \pm 0.01$  g in fish fed Sc3, to  $0.03 \pm 0.00$  g in fish fed diets Lf1 and LfSc2. Increase in spleen weight was noticed in fish fed diets containing *L. fermentum* and *S. cerevisiae* separately, as the level of inclusion increased. The weights of intestine were discovered to range from  $3.19 \pm 0.01$  g in Lf2 to  $2.24 \pm 0.02$  g in LfSc1. The weights of skin and intestine of the experimental fish varied significantly ( $P < 0.05$ ) among the treatments. The weights of kidney in fish fed with diets Lf1, Lf2, LfSc2 and LfSc3 were not significantly different ( $P > 0.05$ ). Fish fed diets containing Lf2 and Sc3, had their liver weights not significantly different ( $P > 0.05$ ).

The enterobacteria counts in the organs of *H. bidorsalis* juveniles fed diets fortified with *Lactobacillus fermentum*, *Saccharomyces cerevisiae*, and their combination during growth study

Table 3 shows the result of microbial load (EBC) in the organs of *H. bidorsalis* juveniles fed fortified diets during growth experiment. The EBC in the kidney ranged from  $3.0 \pm 0.15$  cfu/mL in LfSc1, to  $3.6 \pm 0.15$  cfu/mL in control. The EBC in the skin, spleen and intestine of the experimental fish varied from  $3.1 \pm 0.07$  cfu/mL (Sc2) to  $4.4 \pm 0.15$  cfu/mL (control);  $3.1 \pm 0.03$  cfu/mL (LfSc3) to  $4.2 \pm 0.12$  cfu/mL (control);  $3.4 \pm 0.12$  cfu/mL (Lf1) to  $5.9 \pm 0.73$  cfu/mL (control) respectively. The EBC showed no significant differences in the kidney, skin, and intestine of the experimental fish fed fortified diets. Liver had the lowest EBC ( $3.0 \pm 0.03$  cfu/mL) in Sc3, while the highest ( $4.1 \pm 0.15$  cfu/mL) was obtained in control. The lowest EBC ( $2.6 \pm 0.33$  cfu/mL) in the gills of the experimental fish were recorded in Lf1, while the highest ( $4.7 \pm 0.43$  cfu/mL) was observed in control.

The total viable count in the organs of *H. bidorsalis* juveniles fed diets fortified with *Lactobacillus fermentum*, *Saccharomyces*

*cerevisiae*, and their combination during growth study

The total viable count of the microbes in the organs of *H. bidorsalis* juveniles fed diets fortified with *L. fermentum*, *S. cerevisiae* and their combination during growth study were presented in Table 4. The TVC in the kidney was highest in control with  $4.2 \pm 0.27$  cfu/mL, while, it was lowest in Lf3, with  $3.40 \pm 0.21$  cfu/mL. The TVC in the skin ranged between  $5.5 \pm 0.19$  cfu/mL in control to  $3.6 \pm 0.07$  cfu/mL in Sc3. Highest TVC in the liver of the experimental fish was obtained in control with  $4.5 \pm 0.20$  cfu/mL, while, the least ( $3.3 \pm 0.09$  cfu/mL) was recorded Sc3. Total viable counts in the gills, spleen, and intestine varied from  $3.0 \pm 0.29$  cfu/mL, in Lf1 to  $5.2 \pm 0.65$  cfu/mL in control;  $3.3 \pm 0.15$  cfu/mL, in LfSc2 to  $4.8 \pm 0.23$  cfu/mL in control; and  $3.6 \pm 0.23$  cfu/mL, in Sc1 to  $5.9 \pm 0.73$  cfu/mL in control. Total viable counts in gills, spleen and intestine showed no significant differences among the treated groups, but, showed significant differences in control.

**Table 3:** The enterobacteriaceae count in the organs of *Heterobranchus bidorsalis* juveniles fed diets fortified with *L. fermentum*, *S. cerevisiae*, and their combination during the experiment

TRT	Kidney	Skin	Liver	Gills	Spleen	Intestine
Lf <sup>1</sup>	$3.2 \pm 0.03^a$	$3.3 \pm 0.10^a$	$3.1 \pm 0.07^a$	$2.6 \pm 0.33^a$	$3.3 \pm 0.20^a$	$3.4 \pm 0.12^a$
Lf <sup>2</sup>	$3.1 \pm 0.03^a$	$3.4 \pm 0.21^a$	$3.1 \pm 0.07^a$	$3.5 \pm 0.13^b$	$3.3 \pm 0.15^a$	$3.8 \pm 0.10^a$
Lf <sup>3</sup>	$3.1 \pm 0.06^a$	$3.2 \pm 0.15^a$	$3.2 \pm 0.09^{ab}$	$3.4 \pm 0.13^b$	$3.4 \pm 0.21^a$	$4.0 \pm 0.29^a$
Sc <sup>1</sup>	$3.2 \pm 0.06^a$	$3.3 \pm 0.09^a$	$3.2 \pm 0.03^{ab}$	$3.3 \pm 0.15^b$	$3.1 \pm 0.07^a$	$3.6 \pm 0.23^a$
Sc <sup>2</sup>	$3.3 \pm 0.13^a$	$3.1 \pm 0.07^a$	$3.1 \pm 0.07^a$	$3.2 \pm 0.07^b$	$3.3 \pm 0.18^a$	$3.8 \pm 0.10^a$
Sc <sup>3</sup>	$3.1 \pm 0.07^a$	$3.2 \pm 0.03^a$	$3.0 \pm 0.03^a$	$3.2 \pm 0.06^b$	$3.2 \pm 0.09^a$	$3.7 \pm 0.21^a$
LfSc <sup>1</sup>	$3.6 \pm 0.15^b$	$3.5 \pm 0.24^a$	$3.4 \pm 0.10^b$	$3.6 \pm 0.10^b$	$3.1 \pm 0.06^a$	$4.0 \pm 0.09^a$
LfSc <sup>2</sup>	$3.1 \pm 0.07^a$	$3.4 \pm 0.19^a$	$3.4 \pm 0.10^b$	$3.3 \pm 0.09^b$	$3.1 \pm 0.06^a$	$3.8 \pm 0.15^a$
LfSc <sup>3</sup>	$3.2 \pm 0.03^a$	$3.3 \pm 0.03^a$	$3.1 \pm 0.03^{ab}$	$3.3 \pm 0.13^b$	$3.1 \pm 0.03^a$	$3.7 \pm 0.21^a$
Ctrl	$3.6 \pm 0.15^b$	$4.4 \pm 0.15^b$	$4.1 \pm 0.15^c$	$4.7 \pm 0.43^c$	$4.2 \pm 0.12^b$	$5.9 \pm 0.73^a$

Means with the same letter along the column are not significantly different ( $p > 0.05$ )

Legend

- Lf1 - Treatment fed diet containing *L. fermentum* 101 cfu/mL
- Lf2 - Treatment fed diet containing *L. fermentum* 102 cfu/mL
- Lf3 - Treatment fed diet containing *L. fermentum* 103 cfu/mL
- Sc1 - Treatment fed diet containing *S. cerevisiae* 101 cfu/mL
- Sc2 - Treatment fed diet containing *S. cerevisiae* 102 cfu/mL
- Sc3 - Treatment fed diet containing *S. cerevisiae* 103 cfu/mL

- LfSc1 - Treatment fed *L. fermentum* and *S. cerevisiae* at 101 cfu/mL
- LfSc2 - Treatment fed *L. fermentum* and *S. cerevisiae* at 102 cfu/mL
- LfSc3 - Treatment fed *L. fermentum* and *S. cerevisiae* at 103 cfu/mL
- Ctrl - Control fed 41.0 % crude protein basal diet
- TRT - Treatments

**Table 4:** The total viable count of microbes in the organs of *Heterobranchus bidorsalis* juveniles fed diets fortified with *L. fermentum*, *S. cerevisiae*, and their combination during the experiment

TRT	Kidney	Skin	Liver	Gills	Spleen	Intestine
Lf <sup>1</sup>	3.7±0.07 <sup>ab</sup>	3.8±0.03 <sup>ab</sup>	3.5±0.13 <sup>ab</sup>	3.0±0.29 <sup>a</sup>	3.8±0.29 <sup>a</sup>	4.0±0.15 <sup>a</sup>
Lf <sup>2</sup>	3.5±0.12 <sup>a</sup>	4.2±0.24 <sup>b</sup>	3.5±0.09 <sup>ab</sup>	3.8±0.23 <sup>a</sup>	3.8±0.27 <sup>a</sup>	3.8±0.10 <sup>a</sup>
Lf <sup>3</sup>	3.4±0.21 <sup>a</sup>	3.6±0.21 <sup>a</sup>	3.6±0.10 <sup>ab</sup>	3.8±0.17 <sup>a</sup>	3.8±0.29 <sup>a</sup>	4.0±0.29 <sup>a</sup>
Sc <sup>1</sup>	3.5±0.23 <sup>a</sup>	3.7±0.09 <sup>ab</sup>	3.6±0.07 <sup>ab</sup>	3.7±0.19 <sup>a</sup>	3.5±0.12 <sup>a</sup>	3.6±0.23 <sup>a</sup>
Sc <sup>2</sup>	3.7±0.12 <sup>ab</sup>	3.6±0.09 <sup>ab</sup>	3.5±0.10 <sup>ab</sup>	3.7±0.12 <sup>a</sup>	3.7±0.17 <sup>a</sup>	3.8±0.10 <sup>a</sup>
Sc <sup>3</sup>	3.5±0.09 <sup>a</sup>	3.6±0.07 <sup>a</sup>	3.3±0.09 <sup>a</sup>	3.6±0.07 <sup>a</sup>	3.7±0.12 <sup>a</sup>	3.7±0.21 <sup>a</sup>
LfSc <sup>1</sup>	3.9±0.06 <sup>ab</sup>	3.8±0.23 <sup>ab</sup>	3.6±0.07 <sup>ab</sup>	3.9±0.12 <sup>a</sup>	3.3±0.10 <sup>a</sup>	4.0±0.09 <sup>a</sup>
LfSc <sup>2</sup>	3.7±0.09 <sup>ab</sup>	3.6±0.18 <sup>ab</sup>	3.6±0.06 <sup>ab</sup>	3.6±0.10 <sup>a</sup>	3.3±0.15 <sup>a</sup>	3.8±0.15 <sup>a</sup>
LfSc <sup>3</sup>	3.7±0.09 <sup>ab</sup>	3.7±0.15 <sup>ab</sup>	3.5±0.09 <sup>ab</sup>	3.5±0.25 <sup>a</sup>	3.5±0.18 <sup>a</sup>	3.7±0.21 <sup>a</sup>
Ctrl	4.2±0.27 <sup>c</sup>	5.5±0.19 <sup>c</sup>	4.5±0.20 <sup>c</sup>	5.2±0.65 <sup>b</sup>	4.8±0.23 <sup>b</sup>	5.9±0.73 <sup>b</sup>

Means with the same letter along the column are not significantly different (p>0.05)

Legend

- Lf1 - Treatment fed diet containing *L. fermentum* 101cfu/mL
- Lf2 - Treatment fed diet containing *L. fermentum* 102cfu/mL
- Lf3 - Treatment fed diet containing *L. fermentum* 103cfu/mL
- Sc1 - Treatment fed diet containing *S. cerevisiae* 101cfu/mL
- Sc2 - Treatment fed diet containing *S. cerevisiae* 102cfu/mL
- Sc3 - Treatment fed diet containing *S. cerevisiae* 103cfu/mL

- LfSc1 - Treatment fed *L. fermentum* and *S. cerevisiae* at 101cfu/mL
- LfSc2 - Treatment fed *L. fermentum* and *S. cerevisiae* at 102cfu/mL
- LfSc3 - Treatment fed *L. fermentum* and *S. cerevisiae* at 103cfu/mL
- Ctrl - Control fed 41.0 % crude protein basal diet
- TRT - Treatments

### Discussion

The study of organ bacteriological dynamics in *Heterobranchus bidorsalis* juveniles fed diets fortified with *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and their combination revealed that lower enterobactericea (EBC) and total bacteria count (TVC) values were observed in the experimental water and organs of *H. bidorsalis* juveniles fed diets fortified with *L. fermentum*, *S. cerevisiae* and their combination, than those observed in control. The reason for this could be attributed to the presence of probionts included in the fortified diets, which were not found in the control diet. The lower EBC and TVC values recorded in the experimental water and the organ indices of *H. bidorsalis* juveniles, when compared with control, could also be related to the antimicrobial properties exhibited by the probionts included in the diets, which inhibited the growth of other microorganisms in the organs and experimental water of *H. bidorsalis* fed fortified diets (Lara-Flores, *et al.*, 2010 and Swarnendu *et al.*, 2010).

This result agreed with Bello *et al.* (2012) who reported lower EBC values in the experimental water used in rearing *Clarias gariepinus* juveniles fed diets containing onion bulb residues (*Allium cepa*) and walnut leaf (*Tetracarpidium conophorum*). This result implies that bacterial load in the experimental water and the organs of *H. bidorsalis* fed fortified diets, were affected by *L. fermentum*, *S. cerevisiae* and their combination at all inclusion levels. This discovery is in tandem with Zapata (2013) and Shalaby *et al.* (2006), who reported lower bacterial load in the water of Nile tilapia (*Oreochromis niloticus*) fed diets containing garlic and chloramphenicol at different inclusion levels.

The organ indices (kidney, spleen, skin, liver, gill and intestine) of the experimental fish were not significantly increased by fortified diets, however the lower liver weight recorded in the fish fed fortified diets as against control, indicated that the experimental fish fed fortified diets were healthy as described by Fox *et al.* (1997) who described organ-somatic indices as the indicators of health, which could

be used to determine the health status of fish. This agreed with Ada and Ayokunle, (2013) who reported reduction in weight of liver of Nile Tilapia (*Oreochromis niloticus*) exposed to gramoxone (herbicide) concentration.

Higher liver weight recorded in control could signify presence of xenobiotics as reported by Montenegro and Gonzalez (2012) who reported higher hepatosomatic index (HI) values in fish *Labrisomus philippi* taken from 2 different sites. Furthermore, Heath, (1995) attributed variation in (HI) values to nutritional status and parasitic infection, it could therefore be inferred from this study that diets fortified with *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and their combination were of good nutritive value.

### Conclusion

Antimicrobial effects of *L. fermentum* and *S. cerevisiae* has resulted in decrease of microbial loads of both the experimental rearing water and fish, the inclusion of these microorganisms as probiotic in fish feed could improve the aquaculture productivity. These microorganisms could therefore be used separately or mixed and their use has been discovered to be safe in aquaculture industry since they are biodegradable and do not have any side effects as drug resistance which are being reported in synthetic antibiotics (Blumenthal *et al.*, 2000).

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## AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa  
Guide for Preparation of Papers  
Notes to Authors

The Editor in Chief  
January 2013

### Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

- Veterinary microbiology, epidemiology
- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
- wildlife management
- Fisheries and aquaculture development
- Food safety and food hygiene
- One health
- Emerging and re-emerging issues in animal resources
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The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese ), by the editors, after acceptance. Full articles submitted in French will also be published in English.

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Authors are invited to submit electronically their manuscripts via attachment only at [bahpa@au-ibar.org](mailto:bahpa@au-ibar.org) in a secured PDF and word format. Manuscript can be sent by post in case of unavailability of internet services (authors should be aware that in this case it will take longer time to be published).

Authors submitting articles to the BAHPA must follow the guidelines in this document. Submissions that deviate from these guidelines will be returned to the corresponding authors for changes and compliance.

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- Usefulness. Manuscripts submitted must help researchers, trainers, educators and policy makers in all regions of Africa improve their effectiveness.
- Rigorous methodology. Manuscripts submitted must be based on valid and reliable information, documentation or sound concepts, empirically, logically and theoretically supported.
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Full papers of original research

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2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided..
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-

repetitive way. Subheadings may be accepted.

8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
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### Sequence of Preparation

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2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
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7. Run "the spell check" and "grammar check" on the entire file before submission using either the UK English or French standard.
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- *Chapter in a Book*: Leach J, 1993. Impacts of the Zebra Mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs of Western Lake Erie. In *Zebra Mussels: Biology, Impacts and Control*, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
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- *Conference Proceedings*: Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis*: Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
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All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names should be italicized in the manuscript.

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