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SHORT COMMUNICATION

CASE STUDY REPORT

THE USE OF PARTICIPATORY EPIDEMIOLOGY TO DETERMINE THE PREVALENCE RATE AND ECONOMIC IMPACTS OF PPR AND CCPP IN TURKANA COUNTY OF KENYA

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Abstract

Participatory epidemiological Research was conducted in Turkana to identify the two most important livestock diseases, and then characterize their incidence and the economic impact. The study was carried out between 12th to 26th September 2011. Semi-structured interviews, guided by checklists were completed with groups of respondents in each of the 16 randomly selected villages (adakars) to collect data on livestock diseases and their impact on the livelihood of the people. Simple ranking techniques, proportional piling exercises and matrix scoring methods were used to collect data on the importance of the diseases identified. Matrix scoring of clinical signs was used to correlate the disease terms provided by the respondents in local language with the scientific names. The research focused on Lomooh or peste des petit ruminants (PPR) and Loukoi or contagious caprine pleuropneumonia (CCPP). Disease impact matrix scoring (DIMS) was used to correlate the diseases to the economic losses, while participatory mapping, time lines and seasonal calendars were used describe the spatial and seasonal distribution of the diseases. Transect drives was used to collect data on the pasture conditions. Lomooh (peste des petits ruminants) was reported to occur in outbreaks with a median morbidity of 65% and a range of 25% to 90% and a case fatality rate median and range of 95% and 75, to 100% respectively. Loukoi (CCPP) on the other hand was described to be an endemic disease known by the community for a long time and had a median morbidity rate of 50% (with range of 39 to 75% and a median and range case fatality rate of 62% and 40 to 85%, respectively). These losses led to reduced income and food insecurity at the household levels. The biggest challenge to livestock farming (which contributed to 75% of the livelihood) was recurrent drought, insecurity and diseases, with CCPP and PPR being considered as having the largest impact. Respondents indicated that these challenges have made people worse off than they were 20 and 10 years ago and more reliant on external food aid.

Keywords: CCPP, Participatory epidemiology, PPR, Small ruminants, Turkana.

UTILISATION DE L'ÉPIDÉMIOLOGIE PARTICIPATIVE POUR DÉTERMINER LE TAUX DE PRÉVALENCE ET LES RÉPERCUSSIONS ÉCONOMIQUES DE LA PPR ET DE LA PPCC DANS LE COMTÉ DE TURKANA AU KENYA

Resume

Une recherche épidémiologique participative a été menée à Turkana dans le but d'identifier les deux maladies animales les plus importantes, et de caractériser ensuite leur incidence et leur impact économique. L'étude a été réalisée du 12 au 26 septembre 2011. Des entretiens semi-structurés, ont été réalisés avec des groupes dans chacun des 16 villages choisis de façon aléatoire (adakars) pour recueillir les données sur les maladies animales et leurs répercussions sur les moyens de subsistance des populations. Des techniques de classement simples, des techniques d'empilement proportionnel et des méthodes matricielles de notation ont été utilisées pour recueillir les données sur l'importance des maladies identifiées. La notation matricielle des signes cliniques a été utilisée pour établir une corrélation entre la terminologie des maladies fournie par les répondants dans la langue locale etla terminologie scientifique. La recherche s'est concentrée sur la Lomooh ou peste des petits ruminants (PPR) et la Loukoi ou pleuropneumonie contagieuse caprine (PPCC). La notation matricielle des impacts des maladies

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(DIMS) a été utilisée pour établir une corrélation entre les maladies et les pertes économiques, tandis que la cartographie participative, les échéanciers et les calendriers saisonniers ont été utilisés pour décrire la répartition spatiale et saisonnière des maladies. Des parcours de transects ontp ermis de recueillir des données sur l'état des pâturages. Il a été signalé que la Lomooh (peste des petits ruminants) survient sous forme d'épidémies avec un taux de morbidité médian de 65% et une fourchette de 25% - 90%, et un taux de létalité médian de 95% et une fourchettede 75%- 100%.Quant à la Loukoi (PPCC),elle a été décrite comme une maladie endémique connue par la communauté depuis longtemps ; et elle avait un taux de morbidité moyen de 50% avec une fourchette de 39%-75%) et un taux de létalité médian de 62%avec une fourchettede40%- 85%. Ces pertes ont conduit à une baisse des revenus et à l'insécurité alimentaire au niveau des ménages. Le plus grand défi pour l'élevage (qui contribuait pour 75% des sources de revenu) était la récurrence de la sécheresse, de l'insécurité et des maladies, la PPCC et la PPR étant considérées comme ayant les plus fortesrépercussions. Les répondants ont indiqué que ces défis ont empiréla situation despopulations par rapport à celle qu'elles vivaientil y a 20 et 10 ans et les ont renduplus dépendantes de l'aide alimentaire extérieure.

Mots-clés : PPCC, Epidémiologie participative, PPR, Petits ruminants, Turkana.

Introduction

Participatory epidemiology (PE) has been used since the 1980s in veterinary surveillance and research especially with the pastoral communities in the developing countries. Participatory epidemiology entails the application participatory tools to study and solve veterinary epidemiological problems (Mariner and Paskin, 2000). Such methods evolved from rapid rural appraisals (RRA) and later developed to participatory rural appraisals (PRA) (Catley, 1999). The method is able to harness existing knowledge in the community and give the stakeholders a bigger role in planning of programs for animal health, disease surveillance and research thus creating a sense of ownership and sustainability of such programs (Jost *et al.,* 2007). Participatory epidemiology recognizes that the livestock owners have a rich wealth of knowledge on problems affecting their livestock and leads to development of disease control programs that are both acceptable to the stakeholder and are effective (Mariner and Paskin, 2000). The PE approach was developed to overcome the constraints in applying the conventional epidemiology in the developing countries, as such research methods are often expensive and logistically complex to implement (Chambers, 1983) and may not be cost effective. In the recent times, participatory disease surveillance (PDS), a form of active surveillance that taps into traditional information networks to monitor and diagnose outbreaks of infectious diseases, was used during the eradication of Rinderpest in the Horn of Africa (Omiti and Irungu, 2010). Participatory disease surveillance has also been used in the surveillance of emerging epizootics such as avian influenza in Africa and Asia (Omiti and Irungu, 2010). The purpose of this study was to collect data on the dynamics and economic impacts of two important sheep and goat diseases in a pastoral setting, using participatory epidemiology tools. The study aimed to demonstrate the importance of utilizing local knowledge to understand livestock diseases and plan actions for veterinary intervention.

Peste des petits ruminants is a highly contagious and infectious viral disease of domestic and wild small ruminants. The disease is now endemic in West, Central, East Africa and parts of Asia were it causes high mortalities in domestic goats (Roeder and Obi, 1999). Contagious caprine pleuropneumonia, an Office Internationale des Epizooties (OIE) listed diseases, caused by Mycoplasma capricolum subspecies capripneumoniae (Mccp), is a severe disease of goats that causes significant socio-economic losses especially in countries in West and East Africa, Middle East, as well as in Pakistan and India, where it is endemic (Jones 1992; Kusiluka *et al.,* 2000; Kusiluka *et al.,* 2007).

This paper describes research that was carried out using PE tools in Turkana County of Kenya, where pastoral and semi-pastoral farming supports 70% of the population as their main socio-economic activity (CBS, 2001). Although the Veterinary Services and non-governmental organizations such as

Vétérinaires Sans Frontières (VSF) Belgium and Terra Nova have implemented several activities to mitigate disease impact (Bett et al., 2009), the animal health status, especially in small stocks, needs to be better understood.

The prevalence rates of the most important disease identified by respondents, peste des petits ruminants (PPR) and contagious caprine pleuropneumonia (CCPP), were studied.

Materials and Methods

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Study area.

Field data were collected during the month of September 2011 in Turkana County. The County borders South Sudan and Ethiopia to the North, Marsabit and Samburu Counties to the East, Pokot County to the South and Uganda to the West. The county has a human population estimated at 450,860 and an area of approximately 77,000 km2 (Central Bureau of Statistics (CBS), 2010).Seventy percent of the residents depend on pastoral livestock rearing, while limited irrigated crop farming is practiced along the banks of rivers Turkwel and Kerio. The County is mostly semi-arid and arid area receiving an annual mean rainfall of below 400 mm (CBS, 2010).

The study was carried out on 16 randomly selected villages namely Nyangaita, Sarmach,

Kaputir, Katilu, Katilia, Morulem, Lopii, Kasuroi, Loboloo, Kalokol, Riokomor, Lorus, Kanukurdio, Lokipoto, Orum and Namuraputh (Figure 1).

Between 10 and 30 respondents were gathered in each selected center using the government chiefs as the community entry points; each group was interviewed to gather data using semi-structured interviews, simple ranking, participatory piling, (Matiner and Paskin, 2000) matrix scoring, disease impact matrix scoring (DIMS) (Bett, 2009), timelines, seasonal calendars (Catley, 2005), transect drives and participatory mapping. Different data gathering tools were applied at different centers. The choice guided respondent's answers and the evolution of the interview as recommended in PRA methodology (Chambers, 1983).

Semi-structured interviews (SSI)

The respondents in each center were gathered at the community's respected meeting place which was usually under a large tree. The research team sat on the same levels with the respondents to avoid creating a barrier to communication. The respondents were interviewed through a translator using open-ended question guided by pre-prepared checklists of topics. The SSI was used to collect information on livelihood, livestock species kept and livestock diseases (Mariner and Paskin, 2000).

Simple ranking method

The respondents were asked to arrange symbols representing the livestock species and diseases mentioned in interview in the order of their importance. The symbols were drawings done on cards or common objects found in the area like stone and pieces of wood (Catley, 1999). This was used to understand the community perception on the importance of their livestock and the challenges they encounter.

Proportional piling

The dividing of piles method was used to estimate the relative morbidity, mortality and case fatality of the various diseases. A hundred pieces of stone of the same size were collected from the ground to represent the total herd of interest and the respondents were asked to divide the pile into two piles representing the animals that were affected with a certain disease and those not affected during the last one year (Figure 2).

The procedure was repeated with all the diseases and the morbidities, mortalities and fatalities estimated. The measures of central tendency and dispersion of the data used were the median and the range of these proportions (Catley, 1999).

Matrix scoring

Matrix was used to understand the local perception of the importance of different clinical signs in the recognition or diagnosis of traditional disease entities. This was adapted from a method described by Catley et al., (2002), where the prominence of a range of

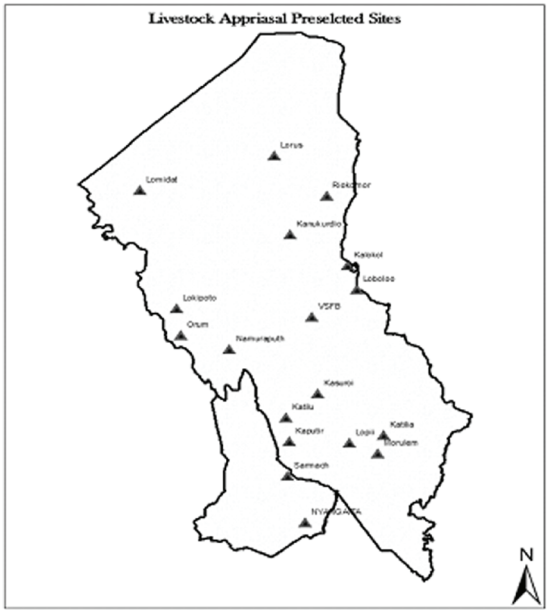

Figure 1: A map showing the sites selected for the participatory study in Turkana County.

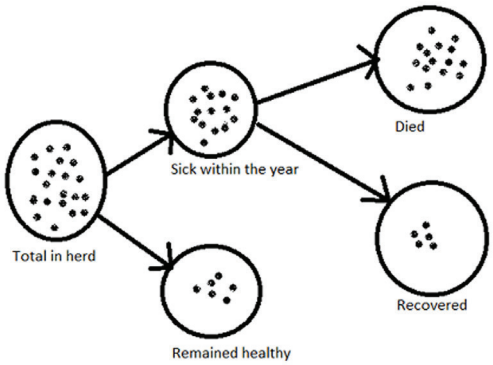

Figure 2: Demonstration of dividing of piles of the counters to give estimates of the morbidity rate, and a further separation to give an estimate of the case fatality rates

clinical signs in the recognition of various local disease concepts described is scored in a matrix format. In this exercise, the diseases that were scored as most important in a proportional piling were represented by the commonly found objects and used to label the rows on a matrix drawn on the ground. A range of clinical signs were represented using objects or drawing on cards and placed on the columns. The respondents were then asked to divide 20 stones down each column according to the relative prominence of the clinical signs in each disease. The respondents were prompted to discuss as a group and agree whether the piling was accurate. The procedure was then repeated for the next disease, and after the matrix was complete, the respondents were asked additional questions to cross-check the scores.

Seasonal calendars

Seasonal calendars were used to understand the seasonal distribution of CCPP and PPR. These were done by identifying the various seasons in the study area, as described by the respondent. The seasons were represented on one axis and the respondents were asked to divide a pile of counters according to the relative occurrence of the disease in the seasons. The procedure was then repeated for the next disease and the respondent were probed with additional open-ended questions meant to elicit further information and to test the accuracy of the piles (Catley et al 2001).

Direct observation of the pasture condition

This was an observational technique to assess the pastures as the research team travelled to the adakars. This was combined with participatory mapping to give a picture of the pasture conditions, which influence the concentration of animals that may create a suitable platform for disease transmission. The direct observations to be made were the pattern of human settlements, distribution and condition of pastures, environmental degradation, watering points and patterns of migration.

Control measures

Various control mechanism were discussed with the respondent, to assess the appropriateness of measures and the farmers are willing to implement together with the veterinary authority. This was done by identifying the disease control measures at the community disposal, during the interviews, then ranking the community preference followed by discussion on the same.

Results.

Livestock kept and disease

The respondents listed goats, sheep,

Table 1: The livestock species ranked in order of their importance per site, using simple ranking method

Division	Sites sampled	Types of livestock in order of rank
Pokot Central	Nyangaita, Sarmach	Cattle, goats, sheep, donkeys Camels and bees
Turkana South	Kaputir, Katilu, Kasuroi	Cattle, goats, sheep, donkeys, camels and bees
Turkana East	Lopii, Morulem, Katilia	Goats, sheep, donkeys
Turkana Central	Lobolo, Kalakol	Goats, sheep and donkeys
Turkana North	Riokomor, Lorus, Kanukurdio	Goats, sheep, donkeys and cattle,
Turkana West	Lokipoto, Olopoi	Cattle, goats, sheep and donkeys
Loima	Namorupoth, Orum	Cattle, goats, sheep and donkeys

Table 2: livestock diseases present in the study area at the time, described clinical signs and the rank in order of importance

Species	Disease described	Clinical manifestations	Rank
Cattle	Lokoi (*CBPP and/or its differentials*)	Labored breathing, coughing, 50% case fatality rates	1
	Lotome (*LSD and/or its differentials*)	Lumps on skin, defoliation	2
	Eyala (*FMD and/or its differentials*)	Salivation, Starling hair coat, limping	3
	Lopid (*L/flukes and/or its differentials*)	Enlarged gall, parasites in hepatic ducts	4
	Lokulup (*grass poisoning*)	Swollen joints, lameness.	5
Goats	Loukoi (*CCPP and/or its differentials*)	Labored breathing, coughing, 50% case fatality rates	1
	Lomooh (*PPR and/or its differentials*)	Lacrimation, nasal discharge, diarrhea	2
	Lotome (*Mange and/or its differentials*)	Defoliation, skin lesions	3
	Etune (*Goatpox and/or its differentials*)	Skin lesion, defoliation	4
	Ngiborok (*Orf and/or its differentials*)	Lips lesions, death in kids	5
	Lopele (*Worms*)	Emaciation, diarrhoea	6
Sheep	Lanyang	Jaundice, yellow urine, death	1
	Loukot	Bloody diarrhea, death	2
	Lomooh (*PPR and/or its differentials*)	Lacrimation, nasal discharge, diarrhea	3
	Emadang (*Ticks infestation*)		4
	Ngilach (*lice infestation*)	Lice, emaciation	5
Camel	Lowala (*Pneumonia*)	Coughing, Nasal discharge	1
	Ekwakoyit (*T. evansi and/or its differentials*)	Anorexia, emaciation Death	2
	Emitina (*Mange*)	Skin lesions	3
Donkey	Emitina (*Mange*)	Skin lesions	1

cattle camels, donkeys and chicken as the main livestock species kept (Table 1).

Livestock diseases

The respondents described and ranked the then current diseases of livestock, where loukoi and lomooh were ranked as the most important diseases in goats (Table 2).

Matrix scoring

Matrix scoring was done to compare the relative importance of a range of clinical signs in lomooh and loukoi,The scoring of clinical signs was consistent with an interpretation of terms where loukoi referred to CCPP, while lomooh referred to PPR.The most predominant clinical signs of lomooh were nasal discharge,

Table 3: Score of the clinical signs for lomooh and loukoi with the median score, and the range

Clinical signs	Lomooh	Loukoi
Lacrimation/ocular discharge	5 (1, 8)	2 (0, 5)
Nasal discharge	8 (3,10)	3 (0, 8)
Swollen head	4 (1, 6)	0 (0, 4)
Fever	2 (1, 3)	0 (0, 4)
Coughing	0 (0, 2)	12 (8, 20)
Emaciation	0 (0, 3)	4 (0, 6)
Sudden death	6 (3, 9)	2 (0, 5)

Table 4: The average (median) and range of the morbidity and case fatalities of Lomooh and Loukoi as generated by the participatory piling exercise.

Disease parameter	lomooh	Loukoi
Morbidity	65% (25%, 90%)	50% (39%, 75%)
Case fatality	95% (75%, 100%)	62% (40%, 85%)

lacrimation/ocular discharge, swollen head and sudden death, while the prominent signs of Loukoi were coughing, nasal discharge and emaciation. Sudden death was not marked, but occurred a few days or weeks after the onset of the clinical signs (Table 3).

Seasonal calendars

The seasonal calendar exercise revealed that the area had two rainy seasons, the long rainy season being from April to July and the short rainy season was noted to be unreliable and ranged from October to November. Lomooh was said to occur in distinct outbreaks causing high morbidity and mortalities, whereas loukoi was occurred endemically throughout the year with the incidences increasing during dry seasons when the goats are concentrated in the dry grazing areas.

Proportional piling

Proportional piling exercise revealed that loukoi was a disease of goats with a morbidity of 50% in the year before and a case fatality rate of 62%, while lomooh had a morbidity rate of 65% and a case fatality rate 95% (Table 4).

Pasture condition

Pasture conditions were assessed using direct observation and participatory mapping. The risk of both lomooh and loukoi was described to be highest in places with good pastures and thus a high concentration of animals. The rainfall pattern mapping correlated closely with the pasture conditions. Pasture and browse conditions were good on the western and southern part of the County, the best senerio being in Chesogon, while the worst scenario was in Kalokol on the east of the county near the Lake Turkana (Plate 1; Table 5; Figure 3).

Timelines and seasonal calendars

Timeline exercises revealed that the environmental degradation was worse at the time of the study than it was 10 and 20 years ago. Present rainfall was also reported to reduced and more unreliable than it was in the past. Livestock numbers were reported to have declined due to frequent droughts, diseases and inter-clan raids. Respondents stated that this has made the community more food insecure and more dependent to external food aid. Seasonal calendars revealed that the livestock diseases especially loukoi and lomooh had their biggest impact at the end of the dry season and at the beginning of a wet season when the livestock body conditions were very poor.

Control measures

Eighty percent of the respondents were willing to present their animals for vaccination at the time of the interview. Twenty

Good browse condition in Chesogon

Poor browse condition in Lokichar Division

Poor browse condition in parts of Loima

Poor browse an pastures in Kalokol Division

Plate 1: A photographic illustration of the browse situation in the area during the time of study

Table 5: Browse condition in the administrative units of the preselected sites.

	Division	Browse Condition	Remarks
1	Chesegon	Good	Very good rainfall reported
2	Segor	Good	Very good rainfall reported
3	Kainuk	Good	Very good rainfall reported
4	Katilu	Good	Good rainfall reported
5	Lokori	Good	Good rainfall reported
6	Lokichar	Good	Fair rainfall reported
7	Kalokol	Poor	Poor rainfall reported
8	Lokitaung	Poor	Poor rainfall reported
9	Kaaling	Poor	Pasture poor in Northern parts

percent of the respondents were unwilling to present their animals for vaccination with some citing increased mortalities after past vaccination exercise, while others doubted the effectiveness of the vaccines used. Forty five percent would have supported a quarantine order to control the two diseases while 55% doubted the rationale of livestock movement as a measure of controlling diseases.

Discussion

Participatory epidemiological tools gave rapid results. Probing indicated that the stakeholders felt that the recorded results accurately reflected their views indicating

a strong ownership of the information generated. Actions arising from such a process would be well supported to the extent that the information provided by pastoralist is used decision-making concerning animal health interventions. This is in agreement with the views of Mariner and Paskin, (2000).

The results of the piling exercises on the morbidity and mortality associated with loukoi and lomooh may overestimate these parameters. The mean morbidity and mortality estimates obtained for loukoi and lomooh would suggest that the annual death loss to these two diseases alone is 31 and 62%, respectively. This is consistent with an overall death loss of 93% for the year.

The community, that has over the years become food aid dependent, tended to associate assessments with the quantification of relief needs and may have exaggerated the morbidity and mortality hoping to attract more assistance. On the other hand, the livestock owners were not asked to report morbidity and mortality in the context of all disease burdens, and this may have biased the result as well. An improved method that has been shown to more reasonable data is to score the morbidity and mortality of all important health problems in one global exercise. In this approach, the respondents are asked to divide one pile of 100 counters into a series of piles each representing the proportion of their flock that falls sick during the course of the year to each of the diseases that have been identified in the interview. A pile is included for that the proportion that was remained healthy during the year as well as a pile for 'other diseases' reflecting uncommon or undefined diseases. Each pile is then divided into those that died from the disease and those that recovered (Catley *et al.,* 2002).

One big advantage of proportional piling is the ability to estimate mortality and morbidity rate without having to ask the population of the animals, conversely this has a disadvantage in that only the median can be used as indicators of central tendency rather than the means because the sub-population sizes are unknown. Means are more amenable to algebraic treatment and are used for further statistical calculations (Kothari, 2004).

This study estimated PPR to have a morbidity rate of 65% and a case fatality of 95% during the previous outbreak (2010/2011), this closely collaborate the expected morbidity of 90 -100% and a mortality rate of between 20 to 90% as described by Roeder and Obi, (1999). CCPP was estimated to have an incident rate of 50% and a case fatality rate of 62% is close to the morbidity rate described by Swai and Neselle, (2010) using participatory methods.

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SURVEY OF BACTERIAL AND PARASITIC ORGANISMS CAUSING DISEASE AND LOWERED PRODUCTION IN INDIGENOUS CHICKENS IN SOUTHERN NYANZA, KENYA

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Abstract

A cross-sectional study was carried out to identify bacteria and parasites that caused disease and lowered productivity in indigenous chickens in Rachuonyo and Migori districts in Southern Nyanza, Kenya. A total of 21 chickens from 11 randomly-selected homesteads, within a group that was recruited into the African Institute of Capacity building and Development (AICAD) project, were used in the study. The chicken-keepers routinely vaccinated their birds against Newcastle disease and were recovering from an outbreak of Gumboro disease which had caused high mortalities. Picking of the chickens for post-mortem examination was by random selection at household level and also geared towards picking those that showed signs of disease. Bacterial isolations were done from pooled oro-pharyngeal and cloacal swabs, and swabs from liver and/or other organs showing pathology. Parasitological isolations were done from skins and gastro-intestinal tracts. Pasteurella and Klebsiella were isolated from cases that were showing respiratory signs, while Salmonella Gallinarum was isolated from liver and spleen of a few birds showing signs of mild peritonitis. Other bacteria isolated, from oro-pharyngeal and cloacal swabs, included: Staphylococcus, Bacillus, E. coli, and Enterobacter. Aspergillus fumigatus was isolated from a case of skin wounds and defeathering. Parasitological isolations included: ascarids, tape worms, flukes, pin worms, tetrameres, stick-tight fleas and scaly-leg mites. These organisms were associated with various pathological lesions.

Since they indirectly cause stress that is associated with increased susceptibility to other diseases and reduction in productivity of the birds, it was found advisable that, in addition to vaccination against the viral diseases, the poultry-keepers exercised regular deworming and dusting of the birds with acaricides, as well as treating the birds whenever they appear sick.

Key words: Indigenous chickens, lowered productivity, bacteria, parasites, Newcastle disease.

ETUDE DES ORGANISMES BACTÉRIDIENS ET PARASITAIRES À L'ORIGINE DES MALADIES ET DE LA BAISSE DE PRODUCTIVITÉ DES POULETSINDIGÈNES DANS LE SUD DENYANZA AU KENYA

Résumé

Une étude transversale a été réalisée pour identifier les bactéries et les parasites à l'origine des maladies et de la baisse de productivité des poulets indigènes des districts Rachuonyo et Migori dans le Sud Nyanza au Kenya. Au total, 21 poulets provenant de 11 ménages choisis de façon aléatoire, au sein d'un groupe du Projet de l'Institut africain pour le développement des capacités (AICAD), ont été utilisés dans l'étude. Les aviculteurs vaccinaient systématiquement leurs oiseaux contre la maladie de Newcastle, et ceux-ci se remettaient d'une épidémie de la maladie de Gumboro qui avait causé de fortes mortalités. Le choix de poulets pour un examen post-mortem a été fait de façon aléatoire au niveau des ménages,

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et ciblait les oiseaux présentant des signes de la maladie.Des isolements de bactéries ont été réalisés à partir d'écouvillons oro-pharyngés et cloacaux et de prélèvements sur le foie et / ou d'autres organes présentant une pathologie. Des isolements de parasites ont été réalisés à partir de peaux et de tractus gastro-intestinaux. Pasteurella et Klebsiella ont été isolés chez des cas montrant des signes respiratoires, tandis que Salmonella Gallinarum a été isolé dans le foie et la rate de quelques oiseaux présentant des signes de péritonite bénigne. D'autres bactéries isolées à partir d'écouvillons oro-pharyngés et cloacaux comprenaient : Staphylococcus, Bacillus, Escherichia coli et Enterobacter. Aspergillus fumigatus a été isolé à partir d'un cas de blessures cutanées et du plumage.

Les parasites isolées comprenaient des ascaris, des vers plats, des douves, des oxyures, des tétramères, des bâtonnets flagellaires et des acariens sources de pattes galeuses. Ces organismes ont été associés à diverses lésions pathologiques.

Etant donné qu'ils causent indirectement le stress associé à une susceptibilité accrue à d'autres maladies et une réduction de la productivité des oiseaux, il est conseillé aux aviculteurs, outre la vaccination contre les maladies virales, de pratiquer un déparasitage régulier et une application d'acaricides sur les oiseaux, ainsi que le traitement des poulets chaque fois qu'ils semblent malades.

Mots-clés : Poulets indigènes, Baisse de la productivité, Bactéries, Parasites, Maladie de Newcastle.

Introduction

Majority of smallholder farmers in sub-Saharan Africa keep chickens, raised under free-range system with very little and inconsistent grain supplementation. Indigenous chickens currently make up more than 80% of the continent's poultry flock[1,2] In Kenya, indigenous chickens are reared by 90% of rural households1. Expanded indigenous chicken production is hampered by several factors including: diseases, predation, poor feeding, housing, disease control and lack of breed selection. Newcastle disease is the most important disease that occurs every year and kills on average 70 – 80% of unvaccinated indigenous chicken flocks[3,4.] Other important diseases of indigenous chickens are: fowl typhoid, Gumboro, fowl pox and worms5.

The present study was conducted to identify bacteria and parasites that cause disease and lowered productivity of indigenous birds recruited into the AICAD project in Southern Nyanza region. These birds were routinely vaccinated for Newcastle disease. It was expected that the results of the study would be used to formulate effective measures to control diseases among the indigenous chicken population in the region, as well as improve their productivity.

Materials and Methods

Study area

The study was conducted in Awendo and Kasipul divisions of Migori and Rachuonyo districts, respectively.

Rongo

The district lies between latitudes 0 ^{0}40' and 0 ^{0}south, longitudes 34^0 and 34^0 50'east. It is 825 km^2 in area6. Altitude ranges between 1135 and 1700 metres above sea level. Temperature ranges between 17 ^{0}C and 25 ^{0}C. Rainfall pattern is bimodal and ranges from 700mm to 1800mm6. The population is estimated at 330,000 with a density of 387 persons per square kilometre7.

Rachuonyo

It lies between latitudes 0^0 15' and 45' south, longitudes 340 25' and 350 east. The district covers an area of 931square km6. Altitude ranges between 1135 to 1600 metres above the sea level and temperatures range between 14 and 25 ^{0}C. Rainfall pattern is bimodal and ranges between 250mm to1000 millimetre6 and the population is estimated at 380,000 with a density of 400 persons per Kilometre[7].

Study chickens

Picking of chickens for post-mortem examination was by random selection and also geared towards picking those that showed signs of disease. A total of 21 chickens from 11 randomly-selected farms were selected (approximately 2 birds per farm). Most birds in the homesteads had died of Gumboro disease. The study birds were of different ages and

sexes, and were on routine Newcastle disease vaccination programme.

Post-mortem examination and sample collection

Post-mortem examination was done following standard procedures and the following samples were collected:-

For bacteriology

1. Pooled oro-pharyngeal and cloacal swabs were taken from each of the birds and put in Stuart's transport medium
2. Swabs taken from any other organ showing pathology (liver, lung, heart, ovary) were taken and put in separate Stuart's transport media

The swab samples were placed in a cool box and transported to the laboratory within 4 to 6 hrs.

For mycology

Skin sample was taken from a case of skin lesions, accompanied by defeathering and placed in a sterile bottle and transported in a cool box to the laboratory.

For parasitology

Whole or half body-skins (depending on size of the bird) and whole gastro-intestinal tracts of the birds were removed and placed in 70% alcohol for preservation.

Bacteriological and parasitological isolations and characterization

Bacteria were isolated and characterized according to8. Parasites, both ecto- and endo-, were characterized as per9. This was done at the Bacteriology and Parasitology laboratories, Department of veterinary pathology, microbiology and parasitology, University of Nairobi. Sampling for Bacteriological studies was purposive and targeted all sampled birds.

Results

P.M. observations

Various pathological lesions were observed when the birds were opened. These included varying degrees of fibrinous pneumonia and/or air sacculitis and some pericarditis; egg peritonitis; yellowish granular substances; pox lesions. Table 1 shows the distribution of these lesions in the two districts studied.

The percentages showing fleas; scaly legs; enlarged/congested spleen (at 33.3%); yolk sac infection; thickened proventriculus with darkened spots, and presence of yellowish granular substances along neck and, in some cases, all over the abdomen (at 16.7%) were higher in birds in Rachuonyo than Migori. It is interesting to note that none of the 15 chickens studied in Migori had scaly legs; also none of them showed the yellowish granular substances seen in some birds in Rachuonyo. Bacteriological isolation of these granules yielded Bacillus species. One chicken in Rachuonyo had diarrhea, while none of the chickens in Migori had diarrhea.

More chickens in Migori (40%) showed liver involvement than in Rachuonyo (33.3%). Also, birds in Migori showed signs of peritonitis and fibrinous pericarditis at 26.7% each; they showed typhlitis, prominent kidney tubules, packed with urates, skin wounds anddefeathering, abdominal hematoma at 6.7% each that were not observed in Rachuonyo.The skin lesions from Migori yielded Aspergillus fumigatus

Sporadic cases of pox were seen in both Rachuonyo and Migori chickens.

Bacterial isolations

Table 2 shows the bacteria types and prevalence isolated from birds studied in Rachuonyo and Migori study sites. Respiratory involvement in both districts was mainly caused by Pasteurella and Klebsiella. Salmonella Gallinarum was isolated from liver and spleen swabs of a few birds showing signs of mild peritonitis in both areas. Other bacteria isolated from both districts included: Staphylococcus, Bacillus and E. coli; they were mainly visceral. Citrobacter, Enterobacter and Streptococcus organisms were isolated only in Migori.; the most isolated one being Streptococcus, at 33.3%. Bacillus and Pasteurella were isolated more in Rachuonyo (66.7% and 50%, respectively) than in Migori (26.7% and 6.7%, respectively). Pure culture Bacillus organisms were isolated from the

Table 1: Prevalence of lesions seen at post-mortem examination of chickens from Rachuonyo and Migori districts

Lesion	Rachuonyo		Migori	
	Number of birds showing lesion	%	Number of birds showing lesion	%
Fleas around eyes	2/6	33.3	2/15	13.3
Scaly legs	2/6	33.3	0/15	0
Signs of jaundice/liver involvement	2/6	33.3	6/15	40
Enlarged/congested spleen	2/6	33.3	3/15	20
Upper respiratory tract infection/pneumonia/air sacculitis	2/6	33.3	3/15	20
Yolk sac infection/fragile ova	1/6	16.7	1/15	6.7
Thickened provetriculus/tetramers	1/6	16.7	1/15	6.7
Yellowish granular substances along neck/all over abdomen	1/6	16.7	0/15	0
Peritonitis – egg/other	0/6	0	4/15	26.7
Fibrinous pericarditis/endocarditis	0/6	0	4/15	26.7
Typhlitis	0/6	0	1/15	6.7
Prominent kidney tubules packed with urates	0/6	0	1/15	6.7
Skin wounds + defeathering	0/6	0	1/15	6.7
Abdominal hematoma	0/6	0	1/15	6.7
Diarrhoea	1/6	16.7	0/15	0
Those also diagnosed of Gumboro disease	4/6	66.7	14/15	93.3

Table 2: Prevalence of bacterial isolates from chickens in Rachuonyo and Migori districts; indicating organs from which isolated

Organism	Rachuonyo		Migori		Organ isolated from
Salmonella (Gallinarum)	1/6	16.7	2/15	13.3	Liver and spleen swab; Peritonitis
Staphyylococcus	1/6	16.7	1/15	6.7	Oro-pharyngeal swab; Liver
Pasterella (multocida)	3/6	50	1/15	6.7	Oro-pharyngeal swab; Respiratory tract
Klebsiella	2/6	33.3	4/15	26.7	Oro-pharyngeal swab; Lung
Bacillus	4/6	66.7	4/15	26.7	Oro-pharyngeal swab; Lung
E. coli	1/6	16.7	6/15	40	Oro-pharyngeal swab; Liver
Citrobacter	0/6	0	1/15	6.7	Oro-pharyngeal swab
Enterobacter	0/6	0	2/15	13.3	Orpharyngeal swab; Liver
Streptococcus	0/6	0	5/15	33.3	Oro-pharyngeal swab; Liver
Unidentified Gram negative coccobacillus	0/6	0	1/15	6.7	Oro-pharyngeal swab; Liver

Table 3: Prevalence of parasitological isolations from Rachuonyo and Migori Districts

Parasite	Rachuonyo		Migori		Where isolated from
	No. of isolates	%	No. of isolates	%	
Ectoparasites					
Knemidocoptes nutants (mite)	2/6	33.3	0/15	0	Scaly legs
Echinophaga gallinacea (stick tight flea)	2/6	33.3	2/15	13.3	Mainly around the eyes
Endoparasites					
Ascarides Ascaridia galli	3/6	50	9/15	60	Small intestine
Heterakis isolonche	4/6	66.7	6/15	40	Caecum
Tetrameres fissipina	1/6	16.7	3/15	20	Proventriculus
Dispharynx nosuta	2/6	33.3	0/15	0	Proventriculus
Tapeworms					
Raillietina echinibothrida	2/6	33.3	0/15	0	Intestine
Flukes					
Echinostoma revolutum	1/6	16.7	0/15	0	Caecum

yellowish granules seen in some Rachuonyo chickens. Generally, bacterial isolations from Migori were at low percentages. Some of the birds had mixed infections.

Fungal isolation

Aspergillus fumigatus was isolated from a skin sample of one chicken presently with signs of defeathering and wounds. Screening of this chicken for mange gave negative results.

Parasitological isolations

Table 3 shows the parasites (and their respective prevalences) identified from birds studied in Rachuonyo and Migori study sites. Most of the birds had worms; most of them showing mixed infestations, including: ascarids, tape worms, flukes, pin worms, tetrameres. Some chickens had high numbers of different types of worms. Neither tapeworms nor flukes were isolated from Migori chickens.

A number of birds had fleas, while a number had scaly legs (including some that were not part of the 15 studied)

Discussion

Results of the chickens studied showed infections with various bacteria, endoparasites, Fungi and infestations by ectoparasites. These organisms were associated with various pathological lesions seen at post-mortem examination. Some birds showed mixed infections of worms, in addition to the bacterial loads; some had lots of worms. Parasites are known to cause stress through nutrient consumption, blood sucking and irritations. The irritations may also cause pathology like enteritis (catarrhal, ulcerative), which could manifest as diarrhea; and dermatitis[10]. Stress in birds is associated with immunosuppression[11, 12].

The severity of other conditions like pneumonia, fibrinous pericarditis, salmonellosis, may be as a result of the Gumboro disease, clinical and/or subclinical, since it destroys immune-competent cells leading to immunosuppression[10]. This may have been coupled with the effect of the heavy parasite burden observed. It needs to be noted, here, that some of the respiratory conditions may have been due to Mycoplasma infections. This study did not pursue Mycoplasma infections due to lack of the special medium needed to grow it.

Apart from immunosuppression, stress caused to the birds as a result of bacterial, endo- and ecto-parasitic heavy burdens reduces the birds' productivity, be it number of off-springs, meat or egg[11, 12]. Thus efforts need to be made

to reduce the stress so as to allow the birds yield more products. Indigenous chickens are normally kept by resource-poor persons in villages and serve as sources of protein and income for the family5. These birds are also utilized in weddings and other ceremonies; they are also given out as presents to visitors. Increasing the numbers of these birds, by keeping them healthy, will increase capacity of these persons both financially and nutritionally. More birds translate into better economic status and better feeding; thus producing a population that is economically enhanced and healthy. Contrary to popular belief, while comparing laying capacity of commercial layers and indigenous hens, using pubic bone spread13 found that, in some cases, the two were at par; there were also cases where the indigenous birds showed higher capacity. This means that, with better handling indigenous hens can give good returns.

Recommendations

Apart from continuing with vaccination against viral diseases, the farmer is advised to:-

1. Deworm the birds at least once every 3 to 4 weeks to control endoparasites
2. Dust the birds with Malamite or other effective powder at least once every 3 to 4 weeks to get rid of ectoparasites
3. Use antibiotics to treat birds that are suffering from bacterial diseases
4. Clean and disinfect areas where birds turn-in-to at night, from time to time, to minimize bacterial/worm loads and possible re-infections.

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THE BENEFITS OF THE PCR-ITS/FILTER PAPER IN THE DIAGNOSIS OF PARASITES AND CHEMORESISTANT TRYPANOSOMES

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Abstract

The most reliable diagnosis method of animal trypanosomoses often used is the microscopic examination for motile trypanosomes. However, the sensitivity of this method remains relatively lower than the classic PCR (Polymerase Chain Reaction). This latter, besides of its high cost, requires some conditions which sometimes are difficult to apply on the field (conservation of samples at +4°C or -20°C). In this study, buffy coat (BCT) specimens were dotted on the filter paper and conserved during 12 months (September 2004 - October 2005) at ambient temperature until their treatment with Chelex® 5%. Samples were tested using unique "pantrypanosomique" PCR with ITS (Internal Transcribed Spacer) primers. This PCR using polyspecific primers indicated parasitological prevalences of 1.2 to 6.2 times higher than those recorded by the microscopic analysis of the buffy coat on the same samples. Beside the mixed infections which could be detected by one PCR reaction, this method could also distinguish Trypanosoma congolense savanna type from Trypanosoma congolense forest type. The gain of sensitivity and the easy conservation of samples in this method could be used for the detection of the chemoresistance in low parasitological prevalence areas.

Keywords: PCR-ITS, filter paper, diagnosis, trypanosomes, chemoresistance.

INTERET DE LA PCR-ITS/PAPIER FILTRE DANS LE DIAGNOSTIC PARASITAIRE ET DE LA CHIMIORESISTANCE DES TRYPANOSOMES

Résumé

La méthode de diagnostic de certitude couramment utilisée dans les trypanosomoses animales est la recherche microscopique de trypanosomes vivants. Cependant, la sensibilité de cette technique reste relativement faible, comparativement à la technique de la PCR (Polymerase Chain Reaction) classique. Cette dernière, en dehors de son coût élevé, exige des conditions (conservation des prélèvements à la température de +4°C ou -20°C) de réalisation parfois difficiles pour des équipes de terrain. Des prélèvements de buffy coat (BCT) réalisés sur du papier filtre ont été conservés pendant 12 mois (Septembre 2004 - Octobre 2005) à la température ambiante jusqu'au traitement au Chelex® 5 %. L'analyse par PCR unique pantrypanosomique à l'aide des amorces ITS (Internal Transcribed Spacer) a été réalisée à partir de ces prélèvements sur du papier filtre. Cette PCR utilisant des amorces polyspécifiques a indiqué des prévalences parasitologiques 1,2 à 6,2 fois supérieures à celles décelées par l'analyse microscopique du buffy coat sur les mêmes échantillons. En dehors des infections mixtes qu'elle a été capable de déceler en une seule réaction PCR, elle a permis de distinguer Trypanosoma congolense type savane de Trypanosoma congolense type forêt. Au regard du gain en sensibilité et de la facilité de conservation des prélèvements, cette technique pourrait être mise à profit dans la détection de la chimiorésistance dans les zones à faible prévalence parasitologique.

Mots clés : PCR-ITS, papier filtre, diagnostic, trypanosomes, chimiorésistance.

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Introduction

Le contrôle des trypanosomoses animales repose généralement sur la chimiothérapie, la lutte contre les glossines qui sont les principaux vecteurs et l'élevage du bétail trypanotolérant. Les deux premières mesures présentent des limites dans le développement de la résistance aux trypanocides et la difficulté à maintenir de manière durable la pression de la lutte. Il devient alors impératif d'assurer la détection et la surveillance continue des trypanosomoses animales et de la chimiorésistance sur le terrain.

De façon courante, le diagnostic de certitude le plus utilisé dans la détection des infections actives de trypanosomoses animales est basé sur la recherche au microscope des trypanosomes vivants. La sensibilité de cette technique est relativement faible par rapport à la technique de la PCR classique (monospécifique) qu'offre la biologie moléculaire. Cette dernière présente de meilleurs atouts de sensibilité et de spécificité (SOLANO *et al.,* 1997). Toutefois, son utilisation en routine est limitée par le coût de la technique, du fait du nombre de tests (3 à 4) à réaliser par échantillon [car chaque couple d'amorces est spécifique d'espèce (DESQUESNES et DAVILA, 2002)] et du problème que pose la conservation des prélèvements.

Des amorces amplifiant les régions intergéniques de l'ADN ribosomial ont été mises au point (CUPOLILLO *et al.,* 1995 ; McLAUGHLIN *et al.,* 1996) et depuis, plusieurs études sur le diagnostic des trypanosomoses par les amorces ITS (Internal Transcribed Spacer) ont été réalisées (DESQUESNES *et al.,* 2001 ; DESQUESNES *et al.,* 2002 ; GEYSEN *et al.,* 2002 ; AGBO, 2003). L'évaluation et la validation récente de ces amorces ITS (MEWONO, 2004 ; SIDIBE *et al.,* 2005 ; SOULEY KOUATO, 2005) pourraient lever la contrainte à une utilisation plus régulière de la PCR dans le diagnostic des trypanosomoses animales, si entre autres, le problème de conservation des prélèvements est maîtrisé.

C'est dans ce contexte qu'a eu lieu la présente étude pour tester des amorces ITS sur des échantillons de « buffy coat » récoltés sur du papier filtre dans l'objectif de contribuer à l'amélioration de la sensibilité des méthodes de terrain utilisées dans la détection de la chimiorésistance des trypanosomes.

Matériel et Méthodes

Echantillonnage, prélèvements et diagnostic

Entre Septembre et Octobre 2004, des enquêtes transversales, utilisant la technique d'examen du « buffy coat » ou Buffy Coat Technique: BCT (MURRAY *et al.* 1977) pour la détection de trypanosomes dans le sang, et la technique de la PCR (Polymerase Chain Reaction) pour la détection et l'amplification d'ADN spécifique, ont été réalisées dans 6 villages de la zone regroupant les provinces du Kénédougou au Burkina Faso et les cercles de Bougouni et Yanfolila au Mali. Un échantillon de bovins par localité a été constitué selon un échantillonnage stratifié aléatoire de façon à ce que chaque troupeau de la localité soit représenté dans l'échantillon proportionnellement à son importance numérique.

Pour chaque animal, un prélèvement de sang a été effectué à la veine jugulaire dans un tube à EDTA (Acide éthylène-diamine-tétraacétique) et placé sous glace avant examen microscopique. Les examens parasitologiques au BCT ont été réalisés sur place, au plus tard dans les deux heures qui ont suivi les prélèvements. Il s'agit dans ce cas, d'une observation microscopique du « buffy coat » extrait des tubes hématocrites, placé entre lame et lamelle (MURRAY *et al.,* 1977). La mesure de l'hématocrite a été suivie de l'identification des espèces de trypanosomes. Pour les prélèvements destinés à la PCR, des « buffy coats » ont été recueillis sur des feuilles de papier filtre N° 4, qui ont été ensuite séchées pendant quelques minutes à l'air libre, puis envoyées au CIRDES (Centre International de Recherche Développement sur l'Elevage en zone Subhumide) pour des analyses de laboratoire. Les échantillons ont été conservés dans des enveloppes ordinaires à la température ambiante et l'abri de l'humidité durant une période d'un an avant les analyses de laboratoire.

Cette étude a été menée en même temps que des enquêtes longitudinales

conduites dans les mêmes localités dans le cadre du projet ILRI/BMZ-2. Au cours de ces dernières enquêtes, 2 lots d'animaux ont été suivis parasitologiquement par la technique d'examen de « buffy coat » (BCT) sur une période de 56 jours en 5 séances d'observation espacées de 2 semaines.

Traitement au Chelex® 5 %

Le traitement au Chelex® a été réalisé en Octobre 2005, soit une année après les prélèvements. Pour chaque échantillon, le papier filtre a été découpé autour du « buffy coat » (sans toucher le buffy coat), puis déposé dans un tube « eppendorf ». 30 µl d'eau distillée stérile ont été additionnés au « buffy coat », puis le mélange a été agité pendant 10 minutes. A ce mélange, 30 µl de Chelex 100® 5% (1g de Chelex 100® dans 5ml d'eau distillée) ont été ajoutés. L'ensemble a été vigoureusement agité et incubé pendant 1h à 56°C. Cette première incubation a été suivie d'une simple agitation puis d'une seconde incubation pendant 30mn à 95°C. Cette seconde incubation a été également suivie d'une simple agitation. Ensuite, la solution a été soumise à une centrifugation à 12 000 tours pendant 2 minutes. Enfin, cette solution a été refroidie et conservée à +4°C ou à -20°C en fonction du délai pour l'utilisation en PCR.

Polymerase chain reaction : PCR

La PCR a été réalisée à l'aide d'un couple d'amorces amplifiant l'ITS1 (Internal Transcribed Spacer) de l'ADN ribosomal (ADNr) des trypanosomes. Il s'agit d'un test PCR unique pantrypanosomique permettant d'identifier en une seule réaction PCR les principaux trypanosomes responsables des trypanosomoses animales (MEWONO, 2004) après migration de l'amplifiat sur un gel d'agarose. Les noms et les séquences du couple d'amorces utilisées sont consignés dans le tableau I.

L'amplification a été effectuée dans un volume réactionnel par point PCR de 10,5 µl contenant: 0,11 µl d'amorce I (0,2 µM); 0,11 µl d'amorce II (0,2 µM); 1,10 µl de tampon 1X (10 mM de tris ; 1,5 mM MgCl2 et 50 mM de KCl); 0,22 µl de chlorure de magnésium (0,5 mM); 0,88 µl de dNTP (ATP + CTP + GTP + TTP) (200 mM); 0,63 µl de BSA (Bovine Serum Albumine à 1,5mg/ml); 6,73 µl d'eau distillée; 0,22 µl de Taq polymérase (1 UI) et de 0,5 µl de solution d'ADN. La réaction PCR a été réalisée à l'aide d'un appareil PTC-100TM (tubes de 0,2 ml) et a comporté : une dénaturation initiale de 5 minutes à 95°C, 35 cycles d'amplification et une élongation finale de 10 minutes à 72°C. Chaque cycle d'amplification a comporté 3 étapes (figure 1): la dénaturation du double brin d'ADN pendant 1 minute à 94°C, l'hybridation des amorces pendant 1 minute à 55°C et l'élongation ou synthèse des brins d'ADN pendant 1 minute et 20 secondes à 72°C.

Chaque amplifiat a ensuite été soumis à une électrophorèse sur gel d'agarose (2%) en présence de marqueurs de taille et de témoins. La migration a été faite à 120 volts pendant 1 heure 30 minutes.

Résultats

Prévalences par BCT et par PCR

Les prévalences parasitologiques obtenues par l'observation microscopique et par la PCR sont consignées dans le tableau II. La PCR utilisant les amorces polyspécifiques a indiqué des prévalences parasitologiques supérieures de 1,2 à 6,2 fois à celles décelées par la microscopie pour un même échantillon. Dans les localités à faible prévalence (<10% au BCT), le rapport des prévalences PCR/BCT est supérieur ou égal à 2 avec des différences significatives au seuil de 5%.

Types d'infection

Les différentes espèces pathogènes de trypanosomes révélées par les deux techniques sont consignées dans le tableau III. La technique classique d'observation parasitologique (BCT) a permis de déceler des infections à Trypanosoma congolense (Tc), et à Trypanosoma vivax (Tv) et une infection mixte (Tc-Tv). Quant à la PCR unique pantrypanosomique, elle a permis d'obtenir des informations plus détaillées sur l'agent causal. En dehors de l'identification des différentes espèces de trypanosomes (Tc, Tv et Tb) et de la détection des infections mixtes (en une seule réaction PCR), elle serait capable de révéler le

type de trypanosome (savane ou forêt) dans le cas des infections à Trypanosoma congolense. La figure 2 représente un gel d'agarose après électrophorèse.

Discussion

Les résultats sur les prévalences sont comparables à ceux obtenus par DESQUESNES *et al.* (1999) qui ont enregistré avec la PCR monospécifique une prévalence 2,5 fois supérieure à celle de la technique parasitologique classique (BCT). En effet, il est généralement reconnu que la méthode de diagnostic microscopique utilisée est la plus pratique sur le terrain. Elle offre néanmoins le désavantage de ne pas déceler les cas de faibles parasitémies (MURRAY *et al.,* 1977 ; HENDRICKX et NAPALA, 1999).

Il ressort de ces résultats que les prélèvements sur du papier filtre pour une PCR utilisant les amorces polyspécifiques présentent un avantage au regard de la sensibilité de la technique, du coût, de la facilité de conservation et d'expédition du matériel biologique. Cet apport de la PCR pourrait être mis à profit dans l'amélioration de la sensibilité de la méthode générale de détection de la chimiorésistance basée sur le traitement et le suivi des infections chez les animaux naturellement exposés. Ceci, surtout dans les zones à faible prévalence où peu de cas sont généralement révélés par la méthode classique de diagnostic microscopique, limitant parfois les analyses statistiques et rendant difficile l'interprétation des résultats.

Le problème de conservation du matériel biologique (prélèvement) pour la PCR pourrait ainsi être résolu par les prélèvements sur du papier filtre. Un suivi parasitologique classique basé sur le diagnostic microscopique pourrait être complété par des résultats de la PCR. Le problème de contamination des échantillons dans le cas de la PCR pourrait bien être résolu par des précautions à prendre lors des manipulations (port de gants, bon séchage des prélèvements sur du papier filtre avant le transport, etc.…).

S'agissant de la détermination des types d'infection, une augmentation du temps de migratrion de l'amplifiat sur le gel d'agarose pourrait permettre une meilleure distinction entre Trypanosoma congolense type savane (Tcs) et Trypanosoma congolense type forêt (Tcf). Sur l'ensemble des observations, la plupart des échantillons diagnostiqués positifs en microscopie ont été confirmés par la technique de la PCR. Les quelques rares cas qui n'ont pas pu être confirmés pourraient s'expliquer par une très faible parasitémie.

Tableau I: Noms et séquences du couple d'amorces TRYP-B

Nom du couple d'amorces	Nom de la séquence	Séquence : (5'-3')
TRYP-B	ITS1C-F	CCGGAAGTTCACCGATATTG
	ITS1B-R	TTGCTGCGTTCTTCAACGAA

Tableau II : Prévalences comparées entre la microscopie et la PCR

Village	**Taille de l'échantillon**	**Prévalence BCT (%)**	**Prévalence PCR (%)**	**Khi-deux (5%)**	**PCR/BCT**
Bandougou	85	31,76	48,24	***	1,52
Diéri	102	31,37	37,25	NS	1,19
Toussiamban-dougou	29	31,03	37,93	NS	1,22
Chobougou	106	2,83	5,66	-	2,00
Faraba	127	8,66	20,47	***	2,36
Sanana	101	4,95	30,69	***	6,20
Total	**550**	**-**	**-**	**-**	**-**
Moyenne	-	15,82	27,64	***	1,75

*** Différence significative

NS : Différence non significative

Tableau III : Espèces de trypanosomes révélés par la BCT et par PCR

Technique	Négatif	Tb	Tcs	Tcs + Tb	Tcs + Tcf	Tv	Tv + Tb	Tv + Tcs	Tcs +Tcf +Tb	Tc	Tc + Tv	Total
BCT	463	0	-	-	-	24	0	-	-	62	1	550
PCR	397	6	93	9	1	35	2	6	1	-	-	550

Tb:T. brucei;Tc:T. congolense;Tcs:T. congolense (savane);Tcf:T. congolense (forêt); Tv:T. vivax

Tableau IV: Protocole d'évaluation de l'effet préventif de l'isométamidium sur 28 ou sur 56 jours

Lots	J_0	J_{28}	J_{56}
Lot test (50 bovins)	- PCV + BCT - ISMM pour tout le lot test - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR
Lot « témoin » (50 bovins)	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR

PCV: Packed Cell Volume (Hématocrite)
BCT: examen parasitologique microscopique du buffy coat
PCR: Polymerase Chain Reaction
ISMM: isométamidium

Tableau V: Protocole d'évaluation de l'effet curatif de l'isométamidium ou du diminazène

Lot	J_o	J_{14}	J_{28}
Lot de 50 bovins	- PCV + BCT - Traitement au trypanocide à tester pour tous les animaux du lot - Prélèvement pour la PCR	- PCV + BCT - Traitement au trypanocide à tester pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Traitement au trypanocide à tester pour les positifs au BCT - *Prélèvement pour la PCR

** Uniquement sur des animaux positifs et traités à J14, et qui se sont révélés négatifs au BCT à J28 ;*
PCV: Packed Cell Volume (Hématocrite)
BCT: examen parasitologique microscopique du buffy coat
PCR : Polymerase Chain Reaction

Au vu des résultats obtenus par la PCR à partir des prélèvements sur du papier filtre, cette technique de diagnostic parasitaire pourrait éventuellement être préconisée surtout dans les zones à faibles prévalences parasitologiques, pour augmenter la sensibilité de la méthode de détection de chimiorésistance basée sur le traitement et le suivi des infections chez les animaux naturellement exposés (TALAKI et al., 2007). Ainsi, les différents protocoles décrits dans les tableaux IV, V et VI pourraient être utiles pour la détection de la chimiorésistance dans les localités à faible prévalence parasitologique (<10%).

Conclusion

La collecte des prélèvements sur du papier filtre pour le diagnostic des trypanosomoses animales par la technique PCR à l'aide des amorces polyspécifiques (ITS) a donné des résultats satisfaisants, avec une sensibilité plus élevée par rapport à la technique de diagnostic microscopique du buffy coat.

Tableau VI: Protocole d'évaluation simultanée de l'efficacité de l'isométamidium et du diminazène sur 28 ou sur 56 jours

Lots	J_o	J_{14}	J_{28}	J_{42}	J_{56}
Lot test (50 bovins)	- PCV + BCT - ISMM pour tout le lot test - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - **Prélèvement pour la PCR	• PCV + BCT • Diminazène pour les positifs au BCT • Prélèvement pour la PCR	• PCV + BCT • Diminazène pour les positifs au BCT • **Prélèvement pour la PCR	• PCV + BCT • Diminazène pour les positifs au BCT • Prélèvement pour la PCR
Lot « témoin » (50 bovins)	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR	- PCV + BCT - Diminazène pour les positifs au BCT - Prélèvement pour la PCR

** Prélèvements facultatifs pour la PCR
PCV: Packed Cell Volume (Hématocrite)
BCT: examen parasitologique microscopique du buffy coat
PCR : Polymerase Chain Reaction
ISMM : isométamidium

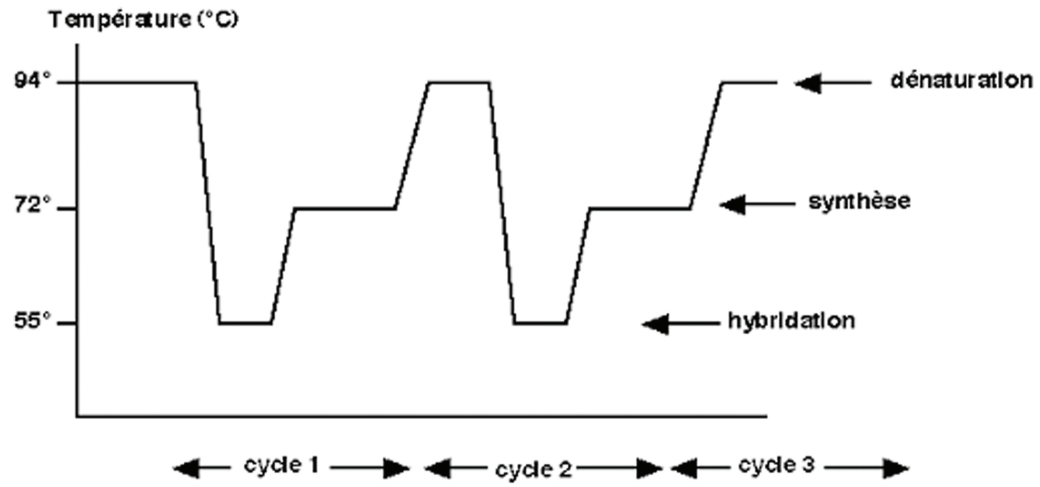
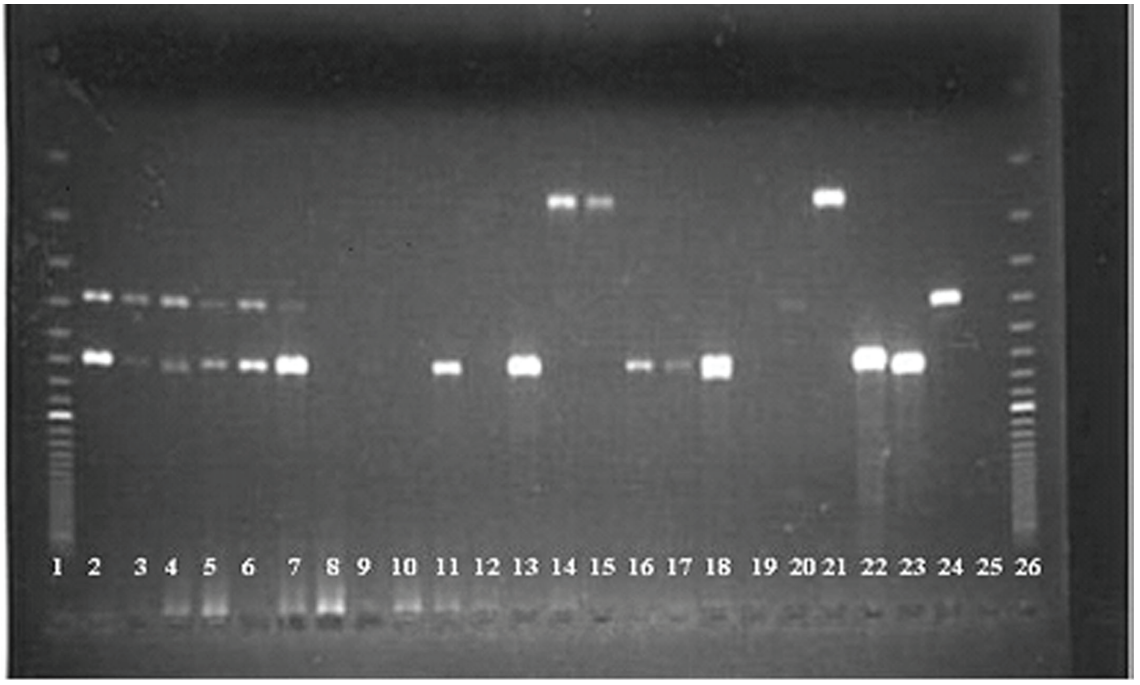


Figure 1 : Cycles d'amplification en PCR



LEGENDE
1 et 26: marqueur de poids
2 à 20 : échantillons
21 : témoin positif Tv
22 : témoin positif Tcs
23 : témoin positif Tcf
24 : témoin positif Tb
25 : témoin positif négatif

Figure 2: Gel d'agarose après migration

Cette méthode de collecte de prélèvement est moins contraignante et rend plus facile la conservation des prélèvements. Par ailleurs, par rapport à la PCR classique, la PCR utilisant les amorces polyspécifiques présente un grand avantage du point de vue coût et gain de temps ; car elle permet de déceler en une réaction PCR les différentes espèces de trypanosomes, même dans les infections mixtes. Cette technique pourrait être mise à profit dans la détection de la chimiorésistance surtout dans les zones à faible prévalence. Une étude comparative des différentes méthodes d'analyse (microscopie, PCR classique et PCR unique pantrypanosomique à l'aide des amorces ITS, en association avec les différentes méthodes de collecte) pourrait être envisagée afin de mieux se situer par rapport au choix d'une technique pour des études bien déterminées. Signalons qu'actuellement des techniques de détection moléculaires de la chimiorésistance des trypanosomes sont mises au point. Bien que ces dernières techniques constituent des outils intéressants dans l'évaluation de la chimiorésistance, elles doivent encore être validées et affinées.

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DETECTION OF RE-EMERGING BOVINE TRYPANOSOMIASIS IN SOUTHERN ZAMBIA BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

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Abstract

In the present study, trypanosome species-specific Loop-mediated isothermal amplification (LAMP) technique, specifically targeting the 18S rRNA gene of Trypanosoma congolense, the repetitive insertion mobile element (RIME) gene of the Trypanozoon subgenus group and the human serum resistant associated (SRA) gene of Trypanosoma brucei rhodesiense, was used to determine the prevalence of bovine trypanosomiasis in the Choma - Kalomo block, an important agricultural area within the Southern province of Zambia. Our data show that out of the 460 cattle sampled, 12.8% (59) were detected to have trypanosomes in their blood by LAMP, suggesting the resurgence of bovine trypanosomiasis in the previously aerial-sprayed Choma-Kalomo block. The majority of those infections were caused by T. congolense. Considering that LAMP is a highly sensitive and specific technique and yet user friendly, this test may in future prove to be instrumental in the routine accurate detection of trypanosomiasis in field samples in resource-limited countries such as Zambia.

Key words: Choma-Kalomo block; LAMP; RIME; SRA; *Trypanosoma brucei brucei*; *Trypanosoma congolense*.

DETECTION DE LA RE-EMERGENCE DE LA TRYPANOSOMIASE BOVINE DANS LE SUD DE LAZAMBIE PAR LA TECHNIQUE LAMP (LOOP-MEDIATED ISOTHERMAL AMPLIFICATION)

Résumé

Dans la présente étude, la technique LAMP (Loop-Mediated isothermal Amplification) spécifique aux espèces de trypanosomes, ciblant spécifiquement le gène 18SrRNA de Trypanosoma congolense, l'élément génétique mobile d'insertion répétitive(RIME) du groupe des sous-genres Trypanozoon et le gène associé à la résistance au sérum humain (SRA)de Trypanosoma brucei rhodesiense, a été utilisée pour déterminer la prévalence de la trypanosomiase bovine dans la région de Choma – Kalomo qui est une importante région agricole située dans la province méridionale de la Zambie. Nos données révèlent que sur les 460 bovins échantillonnés, la technique LAMP a détecté des trypanosomes chez 12,8% (59), ce qui fait penser à la résurgence de la trypanosomiase bovine dans la région de Choma – Kalomo antérieurement soumis à une pulvérisation aérienne. La majorité de ces infections étaient causées par T. congolense. Etant donné que la technique LAMP est très sensible et spécifique mais aussi facile à utiliser, elle pourrait à l'avenir s'avérer très utile dans le dépistage systématique et précis de la trypanosomiase dans les échantillons de terrain, dans les régions aux ressources limitées comme la Zambie.

Mots-clés: Choma-Kalomo; LAMP;; trypanosoma brucei brucei; trypanosoma congolense.

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Introduction

Bovine trypanosomiasis is one of the major constraints to sustainable livestock agriculture development in sub-Saharan Africa (SSA), especially in the traditional sector, which in the case of Zambia accounts for about 80% of the country's cattle population. The annual direct production losses due to bovine trypanosomiasis alone is in excess of US$10,000 million in the 37 tsetse-infected SSA countries (Hursey and Slingenberg, 1995). The majority of the cattle owned by resource-limited farmers are found in tsetse infested areas and are at risk of contracting trypanosomiasis (Coetzer *et al.,* 1994).

Zambia lies within the 'common fly belt' shared together with Malawi, Mozambique and Zimbabwe, in which Glossina morsitans morsitans (*G. m. morsitans*) and *G. pallidipes* are the most abundant tsetse fly species (Van den Bossche and Vale 2000). The Zambian part of the common fly belt is mainly centered on the Luangwa valley and further extends to the Zambezi valley and the adjacent areas. The common fly belt has a large population of wildlife in game parks bordered by human habitats and domestic animals (Symeonakis *et al.,* 2007). The Choma-Kalomo block is an area of approximately 4,500 km2 adjacent to the common tsetse fly-belt closer to the Kafue National Park (Lovemore, 1989). Livestock production is one of the livelihoods of peasant farmers in addition to crop production in the Choma-Kalomo agricultural block. However, vector-borne diseases such as trypanosomiasis theileriosis, anaplasmosis, babesiosis and heartwater negatively affect livestock production.

Diagnosis of Bovine trypanosomiasis remains unsatisfactory in most resource-limited countries in Africa, resulting in poor control strategies of the disease. Currently, the commonly used diagnostic tools include traditional parasitological and serological methods. Loop-mediated isothermal amplification (LAMP) is a new molecular diagnostic method that rapidly amplifies target DNA under isothermal conditions (Notomi *et al.,* 2000). LAMP has recently been developed for use in the diagnosis of several pathogens including animal pathogenic African trypanosomes (Kuboki *et al.,* 2003; Thekisoe *et al.,* 2005; 2007) and human pathogenic African trypanosomes (Njiru *et al.,* 2004; 2008). Unlike other molecular techniques such as the polymerase chain reaction (PCR) which require costly equipment and highly skilled manpower, LAMP is relatively cheaper, requiring only a heating block or water bath and is easier to use. Furthermore, LAMP assays may be conducted within an hour and the products may be visualized by naked eyes (Notomi *et al.,* 2000).

This study was carried out to determine the prevalence of bovine trypanosomiasis in Choma-Kalomo block located in the southern province of Zambia in which tsetse flies were previously eradicated. In 1987, this area was aerial-sprayed under the Regional Tsetse and Trypanosomiasis Control Programme (RTTCP). The overall objective of the RTTCP of Malawi, Mozambique, Zambia and Zimbabwe was to eradicate the tsetse flies from a discrete common fly-belt of approximately 322,000 km2 within the Zambezi valley and the adjacent areas in order to control trypanosomiasis (Connor, 1989). This successfully eradicated the tsetse flies in the area and led to improved cattle productivity and associated agricultural activities (Connor, 1989). Unfortunately, recent field reports from within the Choma-Kalomo block indicate a re-surgence of trypanosomiasis (Personal communication, Silawve, Southern Province Principle Biologist; Soko, Choma district Veterinary Officer). In this regard, there was need to conduct a systematic study to determine the prevalence of re-emerging trypanosomiasis in that important agriculture region.

Materials and Methods

Study area

A cross sectional study was carried out in the Choma-Kalomo agricultural block (Fig. 1) of the Southern province of Zambia in December 2009. The Choma-Kalomo block, approximately 4,500 km^2 in size, is an agriculture zone which incorporates fairly large parts of Choma, Kalomo and a small section of Namwala and Itezhi tezhi districts. A total of

13 sampling sites were identified. Geo-spatial references of the selected crush pens were recorded (Table 1).

Sample collection

The number of cattle to be sampled was determined as described by Cannon and Roe (1982), with an estimated trypanosomiasis prevalence of 1%, confidence level of 95% and total cattle population of 75,000. This gave a minimum of 299 heads of cattle to be sampled. However, a total of 460 heads of cattle were sampled.

A simple random sampling method was applied to systematically select animals at each of the 13 crush pens. From each animal, blood was collected from the superficial ear vein into two heparanised microhaemetocrit capillary tubes. The capillary tubes were sealed with "cristaseal" (Hawksley, UK) and centrifuged immediately in a haematocrit centrifuge. The buffy coat and uppermost layer of red blood cells of centrifuged heparanised microhaemetocrit capillary tubes of each specimen were extruded onto a labelled filter paper (Whatman no. 1, Whatman®) as buffy coat spot for extraction of trypanosomal DNA. The buffy coat spots were dried and kept in zip lock plastic bags containing silica gel at -20^{0}C until DNA extraction. The above procedures were done in the field at the sampling site.

DNA extraction

DNA was eluted from three punched paper disks of dried buffy coat spots according to the modified methanol fixation method described by Johanson *et al.* (2009). The eluted DNA was stored at -20^{0}C until use.

Loop-mediated isothermal amplification

A LAMP reaction of 25 µl was performed using a Loopamp DNA Amplification Kit (Eiken Chemical, Tochigi, Japan) and the parasite DNA as template, according to the manufacturer's instructions, with minor modifications. Briefly, 3 µl of template DNA was added to a 22 µl master mix containing 12.5 µl of reaction buffer (40 mM Trish-HCl (pH 8.8), 20 mM KCl, 16 mM $MgSO_4$, 20 mM $(NH_4)2SO_4$, 0.2% Tween 20, 1.6 M Betaine, 2.8 mM of each dNTP), 1 µl of Bst DNA polymerase enzyme (provided in the Loopamp DNA Amplification kit), 1 µl Loopamp fluorescent detection reagent (Eiken Chemical, Tochigi, Japan), 2 µl primer mix and 6.5 µl distilled water. This study made use of the recently designed primers specifically targeting the 18S rRNA gene of Trypanosoma congolense (Thekisoe et al., 2007), the repetitive insertion mobile element (RIME) gene of the Trypanozoon subgenus group (Njiru *et al.,* 2008a) and the human serum resistant-associated (SRA) gene of Trypanosoma brucei rhodesiense (Njiru *et al.,* 2008b), respectively. The primer mixes were made as follows: (i) CON2 18s rRNA gene of T. congolense (BIP and FIP at 40 pmol each, F3 and B3 at 5 pmol each); (ii) RIME gene of Trypanozoon subgroup (FIP and BIP at 40 pmol each, Loop F and Loop B at 20 pmol each, F3 and B3 at 5 pmol each); (iii) Furthermore, all RIME-LAMP positive samples were tested for T. b. rhodesiense targeting the SRA gene (FIP and BIP at 40 pmol each, Loop F and Loop B at 20 pmol each, F3 and B3 at 5 pmol each). RIME-LAMP positive and SRA-LAMP negative samples were considered to be Trypanosoma brucei brucei. The reaction mixture was incubated at 640C for 30 minutes in a heat block (Dry Thermounit DTU 1B, TAIEC Co., Saitama, Japan) and then at 950C for 2 minutes to terminate the reaction. The LAMP products were visualized using a transilluminator (WD, H19, Good design award Co., Japan).

Data analysis

Data was stored in basic excel format for easy handling and storage. The data was transferred to Stata / SE 8.0 (©1994 – 2003. Stata Corporation) for statistical analyses. Independent sample t-test was used to determine the association between continuous variables, while fisher's exact test was used to determine the association between categorical variables. P values < 0.05 were considered statistically significant.

Results

As shown in figure 2, the LAMP positive samples had bright green fluorescence colour when visualized under the transilluminator and could clearly be distinguished from the negative ones. The LAMP method revealed an overall

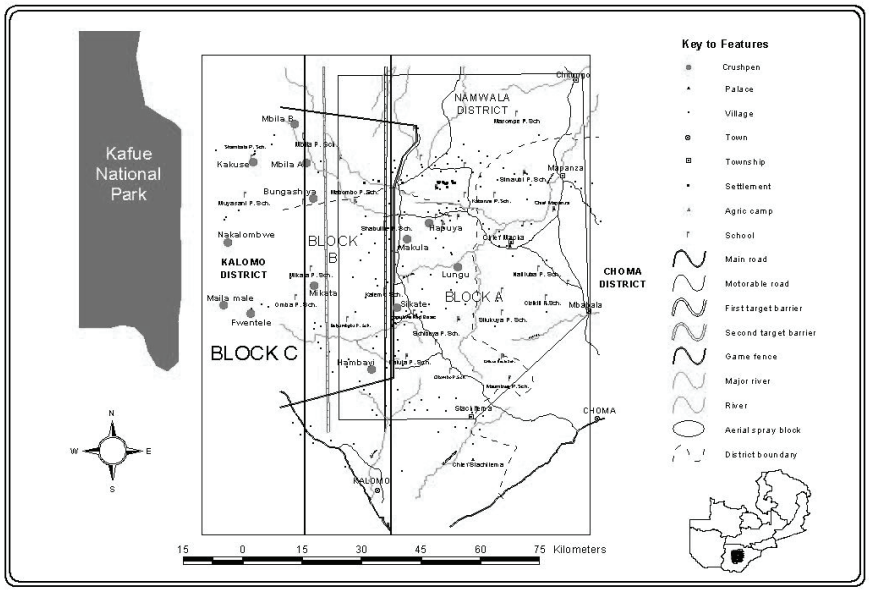

Figure 1: The Choma-Kalomo agricultural block and the thirteen sampling site, crush pens (red spots). The Choma-Kalomo block is an area of approximately 4,500 km2 adjacent to the common fly-belt closer to Kafue National Park, Zambia. It incorporates fairly large parts of Choma, Kalomo and small sections of Namwala and Itezhi tezhi districts and is an important agriculture area.

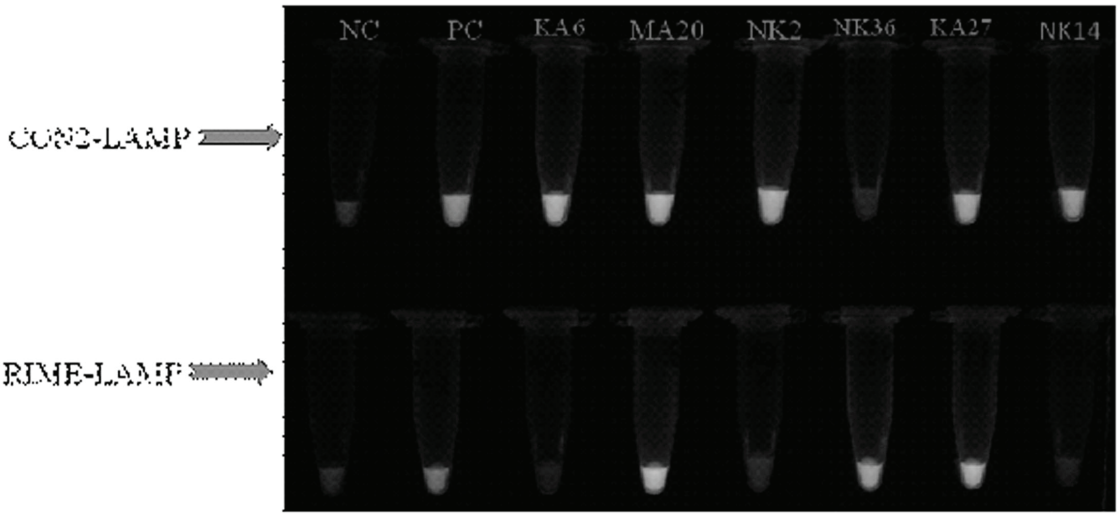

Figure 2: Visual appearance of representative results for Trypanosoma congolense specific (CON2)-LAMP (upper row) targeting 18S rRNA gene and the repetitive insertion mobile element (RIME)-LAMP (lower row) specific for Trypanozoon subgroup. Loopamp® Fluorescent detection reagent was added to the reaction mixture at the beginning of the assay. The reactions were incubated at 64oC for 30 minutes. Positive samples exhibit a bright fluorescent green colour when visualized under the transilluminator. NC: Negative control (distilled water); PC: Positive control (T. congolense for CON2-LAMP; Trypanosoma brucei brucei for RIME-LAMP); KA: Kakuse; NK: Nakalombwe; MA: Mbila A; numbers 2, 6, 14, 20, 36, 27 signify sample identity.

Table 1: Location and geo-spatial references of selected crush pens as sampling site

District	Veterinary camp	Crush pen name	Geo-spatial reference	
			Latitude	Longitude
Choma	Lungunya A	Lungu	S: 16.45846	E: 026.23309
		Hapuya	S: 16.27259	E: 026.31499
		Sikaate	S: 16.38766	E: 026.48587
		Makula	S: 16.38764	E: 026.43072
Itezhi tezhi	Basanga	Mbila A	S: 16.17338	E; 026.31499
		Mbila B	S: 16.22934	E: 026.33706
		Kakuse	S: 16.38676	E: 026.49305
Kalomo	Lungunya B	Hambayi	S: 16.43545	E: 026.38589
		Bungashiya	S: 16.36614	E: 026.14082
	Nkanda zovu	Mikata	S: 16.46427	E: 026.30394
		Fwentele	S: 16.55185	E: 026.13389
		Nakalombwe	S: 16.38044	E: 026.15285
		Maila Male	S: 16.49340	E: 026.15802

(Source: RTTCP, Zambia, 2002)

trypanosome infection rate of cattle from the Choma-Kalomo agricultural block of 12.8% (95% CI: 9.7 –15.8%), with infections being mainly caused by T. congolense 9.5% (95% CI: 2.0 – 17.0%) while 2.4% (95% CI: -1.5 – 6.3%) of the animals were infected with T. b. brucei. In addition, 0.9% (95% CI: -1.5 – 13.3%) animals had mixed-infections of T. congolense and T. b. brucei. No T. b. rhodesiense was detected.

When the disease prevalence was considered in individual crush pen (sampling sites), it was observed that only 6 out of the 13 crush pens recorded positive cases of bovine trypanosomiasis as follows: Nakalombwe 53.5% (95% CI: 39.0 – 68.4%), Kakuse 51.2% (95% CI: 36.3 – 66.1%), Mbila A 36.0% (95% CI: 17.2 – 54.9%), Fwentele 14.3% (95% CI: -11.6 – 40.2 %), Bungashiya 7.1% (95% CI: -2.4 – 16.6 %) and Maila Male 4.9% (95% CI: -1.7 – 11.5 %) (Fig. 3).

Discussion

Trypanosomiasis is a tsetse-transmitted disease affecting both man and livestock in sub-Saharan Africa. This disease has serious socio-economic implications such that it remains a major obstacle to overall development in the affected regions (Hursey and Slingenberg, 1995). Because of the absence of an effective vaccine against the disease due to antigenic variation, trypanosomiasis control is achieved by either targeting the parasite or tsetse vector (Namangala, 2012). An attempt to eradicate tsetse flies in the Choma-Kalomo agricultural block was achieved through aerial spraying in 1987, which in turn resulted in improved animal and crop productivity (Connor, 1989). In the present study, an attempt was made to investigate the prevalence of bovine trypanosomiasis in the previously aerial-sprayed Choma-Kalomo agricultural block by means of a trypanosome-specific LAMP test. We report a 12.8% overall prevalence of trypanosomiasis, suggesting a resurgence of the disease in the Choma-Kalomo agricultural block. Moreover, the presence of tsetse flies in the Choma-Kalomo block was observed during the sampling exercise (unpublished observation).

The prevalence of bovine trypanosomiasis differed substantially between sampling sites within the Choma-Kalomo agricultural block. More importantly, trypanosome infections were generally only detected in sampling sites that were within close proximity to the Kafue National Park. To that effect, the highest prevalence of bovine trypanosomiasis recorded by LAMP was at Nakalombwe (53.5%) and Kakuse

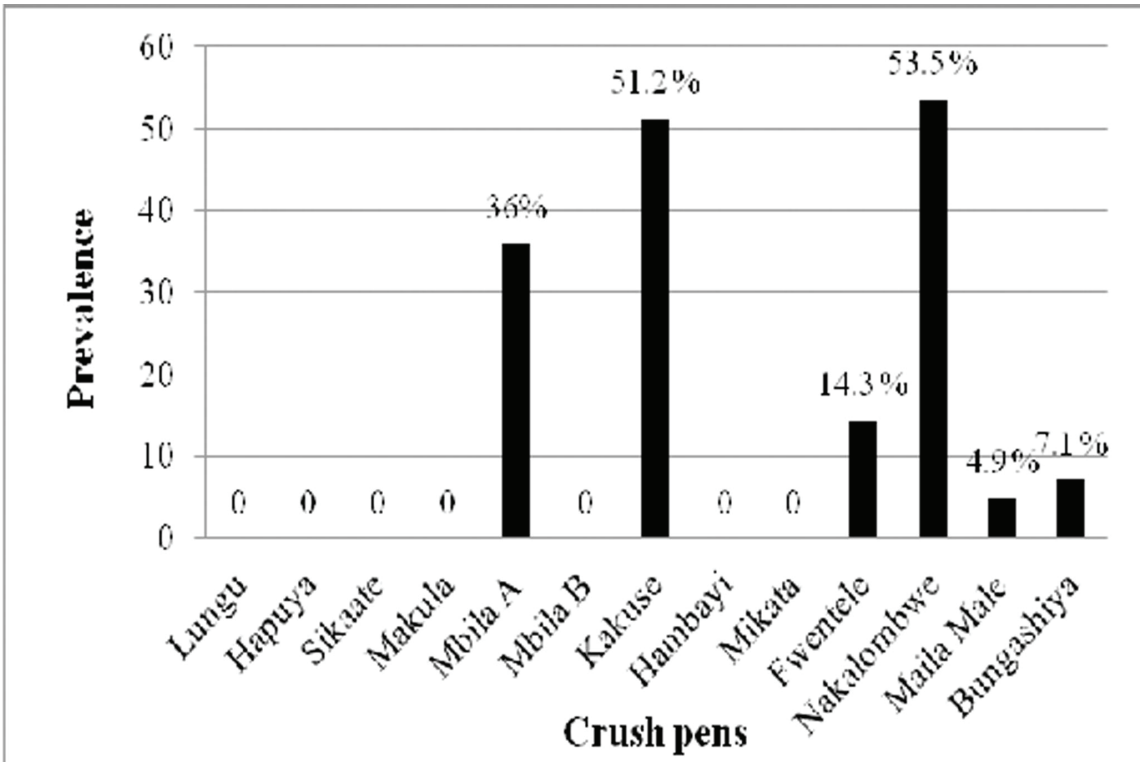

Figure 3: Determination of the distribution of trypanosome infected cattle across crush pens. The prevalence of trypanosomiasis in specific sampling sites within the Choma-Kalomo agricultural block was determined by LAMP tests specific for T. congolense, Trypanosoma brucei brucei and Trypanosoma brucei rhodesiense.

(51.2%), respectively, which were closest to the Kafue National Park. As the distance from Kafue National Park increases (see Fig. 1), the prevalence of the disease tended to decrease (36% in Mbila A; 14.3% in Fwentele; 7.1% in Bungashiya; 4.9% in Maila Male). In sampling sites that were much further away from the park, no infections were recorded. Considering that trypanosomes are tsetse-transmitted parasites, this observation may not be surprising as the tsetse density is expected to be higher within and in close proximity to the Kafue National Park which also harbour large populations of wildlife reservoirs (Symeonakis *et al.,* 2007). According to Van den Bossche and Vale (2000), tsetse flies are more abundant in areas where livestock occupy the edge of tsetse-infested wildlife zones such as the Kafue National park. Our study revealed that the majority of the recorded trypanosome infections in cattle in the Choma-Kalomo agricultural block were caused by *T. congolense*. This is in conformity with previous reports in which *T. congolense* was documented to be the most common trypanosome species affecting cattle in southern Africa (Van den Bossche and Vale, 2000; Simukoko et al., 2010). Furthermore, Simukoko et al. (2007) reported that *T. congolense* accounted for about 34% of bovine trypanosomiasis in Eastern province of Zambia. *Trypanosoma congolense* and *T. vivax* are highly virulent parasites that cause debilitation, loss of productivity and death in cattle and other domestic livestock (Moloo *et al.,* 2008). To that effect, most of the *T. congolense* infected cattle in the present study were debilitated and anaemic. On the other hand, *T. b. brucei* and *T. b. rhodesiense* are thought to be low pathogenic to domestic livestock such as cattle (Ochan, 2004; Njiru *et al.,* 2004).

In conclusion, our data confirms the re-surgence of *trypanosomiasis* in the previously aerial sprayed Choma-Kalomo agricultural block. This was achieved through the use of trypanosome species-specific LAMP. Considering that LAMP is a highly

sensitive and specific technique and yet user friendly (simpler, cheaper, quicker and that LAMP products may be visualized by naked eyes), this test may in future prove to be instrumental in the routine accurate detection of such infections as trypanosomiasis in field samples in resource-limited countries such as Zambia. Future studies should investigate the prevalence of pathogenic trypanosomes in tsetse flies caught within the Choma-Kalomo block so as to give an indication of the risk of contracting the disease in the area.

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SEROLOGICAL SURVEY OF NEWCASTLE DISEASE AND INFECTIOUS BURSAL DISEASE IN BACKYARD BIRDS IN SUDAN

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Abstract

A serological survey on the prevalence of antibodies against Newcastle disease virus (NDV) using haemagglutination inhibition test and infectious bursal disease virus (IBDV) using the agar gel immunodiffusion (AGID) test, was carried out in non vaccinated birds raised under backyard management system in 14 States of Sudan. A total of 910 serum samples were collected (843 chickens, 45 pigeons and 22 ducks) during 2009 to 2010. The overall seroprevalence of NDV in backyard chickens was found to be 41.8% with mean antibody titre 2.75 log2. The highest prevalence rates (68.9) was recorded in Northern State and the lower (28.9) in Blue Nile State with significant variation ($P<0.05$). The prevalence of NDV in other bird species was 23.88% (22.2% of pigeons, (27.3) % of ducks) with no significant variations between species. IBDV antibodies were found in 30.6% backyard chickens, the significantly highest value (31.5) was observed in South Darfur State and lowest (0) in Red sea State while the tested pigeons and ducks were reacted negative. The findings of the present study indicated that NDV and IBDV were endemic and widely distributed in backyard areas of Sudan which should be incorporated in the control strategies.

Key words: backyard birds, Newcastle disease, Infectious bursal disease, antibodies, Sudan

ETUDE SÉROLOGIQUE DE LA MALADIE DE NEWCASTLE ET DE LA BURSITE INFECTIEUSE CHEZ LES OISEAUX DE BASSE-COUR AU SOUDAN

Résumé

Une enquête sérologique sur la prévalence des anticorps antiviraux de la maladie de Newcastle (NDV) utilisant le test d'inhibition de l'hémagglutination et de la bursite infectieuse (IBDV) utilisant le test d'immunodiffusion en gélose (AGID) a été réalisée chez des oiseaux non vaccinés de basse-cour dans 14 États du Soudan. Au total, 910 échantillons de sérum ont été prélevés (843 poulets, 45 pigeons et 22 canards) de 2009 à 2010. On a noté que la séroprévalence globale du virus de la maladie de Newcastle (NDV) chez les poulets de basse-cour était de 41,8%,avec un titre d'anticorps moyen de 2,75 log2. Les taux de prévalence les plus élevés (68,9) ont été enregistrés dans l'État du Nord et le taux le plus faible (28.9) dans État du Nil bleu, avec une variation significative ($P <0,05$). La prévalence du NDV chez d'autres espèces d'oiseaux était de 23,88% (22,2% des pigeons, 27,3% des canards), sans variations significatives entre les espèces. Des anticorps anti IBDV ont été identifiés chez 30,6% des poulets, la valeur la plus élevée (31,5) ayant été observée dans le sud de l'État du Darfour et la plus faible (0) dans l'État de la Mer Rouge, tandis que les pigeons et les canards soumis au test ont montré une réaction négative. Les résultats de la présente étude ont indiqué que les virus NDV et IBDV étaient endémiques et largement répartis dans les régions pratiquant le système de basse-coursau Soudan, et cela devrait être intégré dans les stratégies de contrôle de ces maladies.

Mots-clés : Oiseaux de basse-cour, Maladie de Newcastle, Bursite infectieuse, Anticorps, Soudan

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Introduction

Traditional poultry production in the Sudan is practiced in rural and periurban areas where conditions permit settled life. The household poultry production system is based on scavenging indigenous domestic chickens accompanied by pigeons, guinea fowls, ducks or turkeys). These backyard birds are usually raised as scavengers in an open yard, scratching and picking on the grounds (Khalafalla *et al.,* 2001).

In Africa it is estimated that 80% of the poultry population is found in traditional production systems and contributed up to 90% of the chickens reared and they supply the bulk of the national requirements of eggs and meat for the urban populations. (Sayda, 2012). Of the approximately 45 million poultry kept in Sudan, 15 million are free range birds (Ministry of Agriculture and animal resources Khartoum state, 2005). These backyard birds play an important role in the provision of animal protein for the population, as both poultry meat and eggs.

The major constraints of household poultry keeping and traditional open house producers are inadequate health care; their major problem is the high incidence of Newcastle disease, and infectious bursa disease (khalafalla *et al.,* 2001).

Newcastle disease (ND) is a contagious and fatal viral disease affecting all species of domestic and wild birds. It was also defined as one of the most important diseases of poultry worldwide (Murphy *et al.,* 1999b). The disease is caused by a virus of a genus Avulavirus belonging to the Family Paramyxoviridae (OIE, 2009). The disease was first isolated and identified in the Sudan in 1962 in a natural outbreak of the disease (Karrar and Mustafa, 1964), the most prevalent strains of the virus identified, were reported to belong to the viscerotropic velogenic pathotype (Khalafalla *et al.,* 1992).

Infectious bursal disease (IBD) is a highly contagious immunosuppressive viral infection of young chickens (3-6 weeks old) causing severe economic and production losses worldwide (Müller *et al.,* 2003), is caused by a virus that is a member of the genus Avibirnavirus of the family Birnaviridae (Murphy *et al.,* 1999a). The first documented evidence of IBD dates back to 1982 (Shuaib *et al.,* 1982). The field reports indicate that ND and IBD seem to be the most important poultry diseases epidemic inflicting high losses every year.

The aim of the present study is to evaluate the prevalence of ND and IBD antibodies in local chickens and other bird species, kept under the traditional back-yard system in Sudan.

Materials and Methods

Study area and blood sampling

The present study conducted in fourteen States in Sudan. (Table1). The sampling was taken from selected flocks which had willingness to participate in the study. The birds were of mixed ages, not vaccinated against diseases, reared under free range or backyard systems. A total of 910 blood samples were collected. Of these, 843 sera were taken from chickens, 45 from pigeons and 22 from ducks. 2-3 ml blood was collected from the wing veins and dispensed in sterile test tubes, left to clot. The serum was separated and transferred into eppendorf tubes, labeled and stored at -20 C0 until tested.

Serology

Haemagglutination Inhibition (HI) test

The HI test was done according to standard procedures (OIE, 2009) to detect NDV antibodies. The antigen used was reconstituted commercial NDV LaSota vaccine. The test was carried out by running twofold dilutions of equal volumes (0.025 ml) of phosphate buffered saline (PBS) and test serum (0.025 ml) in V-bottomed microtiter plates. Four haemagglutination units of virus / antigen was added to each well and the plates were left at room temperature for 30 minutes. Finally, 0.025 ml of 1% chicken red blood cells (RBCs) was added to each well after gentle mixing, allowed to settle for 30 minutes at room temperature. The HI titer was read from the highest dilution of the serum causing complete inhibition of the 4 HA of antigen.

Agglutination was assessed by titling the plates. Only those wells in which the RBCs stream at the same rates as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) were considered to show inhibition. An HI titre of 1:16 or greater was regarded as positive (OIE, 2009).

Agar Gel immunodiffusion (AGID) test

Sera were examined for IBD antibodies using the AGID test as described by Cullen and Wyeth, (1975). Standard IBD antigen for AGID (Intervet Ineternational B.V. Boxmeer, Holland) was used. The appearance of precipitin lines between the central wells filled with standard IBD antigen and any of the test sera in the peripheral wells was regarded as positive results.

Statistical analysis

Collected data was analyzed using software package Statistix 9. Chi-square analysis was used to compare the proportions of antibodies of chickens between States and the overall proportion. In all chi-square tests a probability level of $P < 0.05$ was considered statistically significant.

Results

NDV antibodies

Out of 843 chicken sera tested, 352 (41.8%) were positive for NDV antibodies. The highest values (68.9) was recorded in the Northern State and the lowest (28.3) in Blue Nile State which was significantly different ($P<0.05$). There was a significant variation ($P<0.05$) in NDV antibodies proportion of the Blue Nile, Sennar, Kassala, River Nile and Northern States. The mean NDV antibody titre was 2.75 $\log_2$, the highest values was recorded in Kassala State and lower in White Nile State. None of the States tested were found to be free from NDV antibodies (Table 1).

As shown in Table 2. the prevalence of NDV in other bird species was 23.88 % (23 out of 67). 13 out of 45 (22.2%) pigeons were positive while 6 out of 22 (27.3%) ducks were positive. There was no significant variations ($P>0.05$) in the proportions between species. The mean antibody titre of pigeons and ducks was 1.24 $\log_2$ and 1.68 $\log_2$ respectively.

IBDV antibodies

A total of 258 out of 843 chicken sera (30.6%) had positive IBDV antibodies. The highest percentage (51.3) was observed in South Darfur State while the lowest (0) in Red Sea State. The proportion of IBDV was significantly different ($P<0.05$) in the States of Khartoum, Gazeera, Blue Nile, Sennar, Red sea, Northern, North Kordofan and South Darfur (table 3).

The tested pigeons and ducks were found negative for IBDV.

Discussion

The data of the present study confirmed that NDV and IBDV antibodies were prevalent in backyard chickens in most parts of the country. These antibodies are most likely due to natural infection as no vaccinations against diseases were practiced in backyard poultry in addition the presence of antibodies is indicative of continues infection pressure. This might be as explained by Zeleke *et al.,* (2005) that the free range management system allows uninterrupted cycle of infection as the virus passes from one another. The relatively higher overall seroprevalence rate of NDV and IBDV antibodies in indigenous chickens may be attributed to a number of factors: the poor sanitary conditions, continuous exposure of chickens to changeable conditions and wild birds, nutritional deficiencies, the absence of vaccination in traditionally managed chickens, and contact of chickens in live bird markets may facilitate the spread of NDV and IBDV. This hypothesis was in agreement of previous report of Sawi *et al.,* (2011).

In the present study, in most of States, the NDV antibody titer was found in different ranges. This could be due to non-intensive rearing system in backyard chickens that resulting in different stages of infection in these chickens. Or attributed to the fact mentioned by Sinha, (1975) that natural infection of chickens with NDV is known to produce high titre than vaccination.

Table 1: Seroprevalence of NDV antibodies in chickens in different States of Sudan &different HI antibody titre(log_2)

State	No. reactors/ No. tested	% +ve	No. of Positive sera at different HI titre									
			$<2^4$	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	2^{11}	Mean HI titre (log_2) ±SE
Khartoum	54/115	47	61	7	5	7	3	20	4	5	3	3.43 ±0.4
Gazeera	9/18	50	9	1	2	1	1	4	0	0	0	3.28 ±0.8
Blue Nile	26/92	28.3*	66	3	5	1	4	8	2	3	0	1.99 ±0.3
White Nile	19/60	31.7	41	3	7	2	1	6	0	0	0	1.9 ±0.4
Sennar	21/72	29.2*	51	3	1	1	0	15	1	0	0	2.11 ±0.4
Gadaref	17/44	38.7	27	4	6	4	1	2	0	0	0	2.11 ±0.4
Kasssla	31/53	58.5*	22	5	4	3	1	10	3	2	3	4.2 5 ±0.5
Red Sea	8/24	33.3	16	1	1	3	0	3	0	0	0	2.1 3 ±0.6
River Nile	29/45	64.4*	16	9	5	3	3	9	0	0	0	3.82 ±0.5
Northern	31/45	68.9*	14	8	4	9	7	3	0	0	0	3.98 ±0.4
North Kordofan	29/77	37.7	48	1	9	8	6	4	0	1	0	2.35 ±0.4
South Kordofan	21/54	38.9	33	1	4	4	4	3	2	3	0	2.74 ±0.5
West Darfur	24/64	37.5	40	4	4	5	1	4	5	1	0	2.5 ±0.4
South Darfur	33/80	41.3	47	4	9	11	4	1	0	2	2	2.75 ±0.1
Total	**352/843**	**41.8**	**491**	**54**	**66**	**62**	**36**	**92**	**17**	**17**	**8**	**2.748517**

Significantly different (P<0.05)
SE=Standard error of the mean

Table 2: Seroprevalence of NDV antibodies in pigeons and ducks &mean HI antibody titre(log_2) in 3 States of Sudan

State	Total No. of samples	Pigeon		Duck	
		No. +ve/ no. tested(%)	Mean HI Titre (log_2) ±SE	No. +ve/no. tested (%)	Mean HI Titre (log_2)±SE
Blue Nile	21	3/7(42.9%)	2±0.5	6/14 (42.9)	2.64 ±0.9
White Nile	19	4/19 (21.1)	1.32 ±0.6	0/0	-
Gadaref	27	3/19 (15.8)	0.89 ±0.9	0/8	-
Total	**67**	**10/45 (22.2)**	**1.24 ±0.4**	**6/22 (27.3)**	**1.68 ±0.6**

SE=Standard error of the mean

Table 3: Seroprevalence of IBD antibodies in chickens in different States of Sudan

State	No. +ve/total no. tested	% of positive
Khartoum	55/115	35.5*
Gazeera	6/18	33.3*
Blue Nile	29/92	31.5*
White Nile	17/60	28.3
Sennar	14/72	19.4*
Gadaref	9/44	20.5
Kasssla	16/53	30.2
Red Sea	0/24	0*

State	No. +ve/total no. tested	% of positive
River Nile	8/45	17.8
Northern	2/45	4.4*
North Kordofan	32/77	41.6*
South Kordofan	19/54	35.2
West Darfur	17/65	35.4
South Darfur	41/80	51.3*
Total	**258/843**	**30.6**

Significantly different (P<0.05)

In this investigation NDV antibodies were detected in tested pigeons and ducks sera. These backyard birds were reared under scavenging system and were allowed to scavenge with backyard chickens in yards and migratory birds. This factor may contribute in transmission of the virus between different species.

It is noted in this study, that chickens in 13 tested States had antibodies to IBDV. But no antibodies were detected in chickens in the Red sea States. This may be due to the low number of sample tested in this State. The result of IBDV in indigenous chickens in the present study suggests that these chickens may play a role in the transmission of IBDV to the commercial flocks.

The current study covered 14 States of Sudan revealing much higher values of NDV seroprevalence when compared to previous similar studies conducted in Sudan, A rate of (16%) NDV antibodies was detected in local chickens in Kordofan region (Elhassan and Kheir, 1989) moreover (12%) was reported in Aldamer province (ElHussein *et al.,* 1995-1996) and (18%) in Gadaref &Khartoum States (Khalafalla *et al.,* 2004). Whereas the rate of NDV antibodies reported here, were closer to the reports from other countries with similar chicken husbandry systems (Agbede *et al.,* 1992; Oyewola *et al.,* 1996; Ashenafi, 2000). The prevalence rates of IBDV antibodies in this investigation, agreed with that of other studies carried out in some parts of Sudan.A percentage 0f (36.3%) was revealed in local chickens in Khartoum and Gadaref States (Egbal, 2002) while Mahasin, (1998) detected (38.7%) in Khartoum North, but in contrary the present data disagreed with earlier investigation of Elhassan *et al.,* (1989) who detect as low as 5.5% prevalence rate in Kordofan region.

The findings of the present study indicate that the most contagious viruses; NDV and IBDV which known to cause high mortalities in poultry were prevalent in birds of backyard farming system in Sudan detected by serology. As the backyard birds were not vaccinated against diseases so the presence of such antibodies was indicative of circulation of those viruses. The perpetuation of the virus in village poultry possesses a potential source of the diseases for the modern poultry sector in Sudan. For this a routine vaccination programs should be implemented in the backyard systems.

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A STUDY ON THE EFFICIENCY OF NATURAL AND SYNTHETIC PROSTAGLANDINS FOR ESTRUS SYNCHRONIZATION IN JENNIES

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Abstract

A study was conducted to determine the efficiency of natural and synthetic prostaglandins in estrus synchronization of jennies. In the experiment, jennies were randomly assigned into Lutalyse (n=5) and Estrumate treatment (n= 7) groups. Ovarian follicular activity was determined ultrasonically. Serum was collected for progesterone assay. The days to estrus, days to ovulation, estrus length, size and number distribution of follicles, and size of the preovulatory follicle were recorded to compare ovarian function. The mean (±SD) number of total follicles was 12.9±3.8 for lutalyse and 13.0±0.5 follicles for estrumate treatments. The mean (±SD) days to estrus, days to ovulation, diameter of the preovulatory follicle and the rate of ovulation for lutalyse groups were 2.3±0.5days, 11.0 ±1.2days, 37.4±2.4mm and 80%, respectively. The same parameters for estrumate treatment were 3.9±0.7 days, 39.5±2.7mm, and 100%, respectively. There was a significant difference ($p<0.05$) in the mean size of the dominant follicles between the two groups; estrumate treatment resulted in the largest dominant follicles. Estrumate also produced shorter days to estrus and days to ovulation ($p<0.05$). In conclusion, both lutalyse and estrumate can be used for estrus synchronization; however, estrumate gives a relatively better response compared to lutalyse for estrus synchronization in jennies.

Key words: estrus synchronization, estrumate, jennies, lutalyse.

UNE ETUDE SUR L'EFFICACITE DES PROSTAGLANDINES NATURELLES ET SYNTHETIQUES POUR LA SYNCHRONIZATION DE L'ESTRUS DE L'ANESSE

Resume

Une étude a été menée dans le but de déterminer l'efficacité des prostaglandines naturelles et synthétiques dans la synchronisation des chaleurs de l'ânesse. Dans le cadre de l'étude, des ânesses ont été réparties de façon aléatoire dans des groupes de traitement par la lutalyse (n = 5) et l'estrumate (n = 7). L'activité folliculaire de l'ovaire a été déterminée par ultrasons. Du sérum a été prélevé pour un dosage de la progestérone. Les jours pré-oestrus, les jours pré-ovulatoires, la durée de l'oestrus, la taille, le nombre et la répartition des follicules, et la taille du follicule pré-ovulatoire ont été enregistrés pour comparer la fonction ovarienne. Le nombre total moyen de follicules était de 12,9 ± 3,8 pour le traitement par la lutalyse et de 13,0 ± 0.5 pour le traitement par l'estrumate. Les moyennes des jours pré-oestrus, des jours pré-ovulatoires, des diamètres du follicule pré-ovulatoire et des taux d'ovulation pour les groupes traités par la lutalyse étaient respectivement de 2,3 ± 0,5 jours, 11,0 ± 1,2 jours, 37,4 ± 2,4 mm et 80%. Les mêmes paramètres pour le traitement par l'estrumate étaient respectivement de 3,9 ± 0,7 jours, 39,5 ± 2,7 mm, et 100%. Une différence significative ($p <0,05$) a été notée au niveau de la taille moyenne des follicules dominants entre les deux groupes : le traitement par l'estrumate a engendré les plus grands follicules dominants. L'estrumate a également causé une diminution du nombre de jours pré-oestrus et pré-ovulatoires ($p <0,05$). En conclusion, la lutalyse et l'estrumate peuvent toutes les deux être utilisées pour la synchronisation oestrale, mais l'estrumate donne une réaction relativement meilleure par rapport à la lutalyse pour la synchronisation oestrale chez l'ânesse.

Mots-clés : Synchronisationoestrale, Estrumate, Anesses, Lutalyse.

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Introduction

Reproductive efficiency is known to be the product of successful estrus detection and conception rates (Delasota *et al.,* 1998). Detection of estrus in group of randomly cycling females is time consuming, laborious and subject to human error (Hafez and Hafez, 2000).All these problems can be possibly solved by synchronization of estrus and ovulation time, so that the females would be in estrus within a predictable time (Blanchard *et al,* 1999; Arthur *et al.,* 2001). In this regard much of the work carried out to date in different species of domestic species is virtually lacking for donkeys. In many parts of Africa where nearly 90% of agricultural operations depend on human muscle, donkeys play an extremely important role in the agricultural sector (Fesseha, *et al,* 1997; Martin-Curran and Smith, 2005). Ethiopia with an estimated 5.2 million heads has the largest donkey population in Africa and the second largest in the world only next to China (Starkey, 2000). As often constrained by prevailing traditional management system, modern donkey production with more option of controlling the reproductive functions and improving performance through breeding have not been properly implemented.

Prostaglandin ($PGF_{2\propto}$) administered as a single intra muscular injection during days of 5 through 17 of the estrus cycle will induce regression of the corpus luteum (CL) and subsequent return to estrus in 36 to 72hours. However, prior to and from days 18 to 21 of the estrus cycle, the CL is refractory to $PGF_{2\propto}$ (Arthur *et al.,* 2001). While much is known about equines, very little is known about donkeys. The presence of species specific factors influencing synchronization of estrus and the variable time to ovulation during estrus, and prolonged duration of behavioral estrus in equines are some of the factors that make synchronization problematic (Kojima *et al* 2000; Bearedn *et al,* 2004; Samper *et al,* 2006; Samper, 2008). Therefore, the objectives of this study were to determine the potential use of estrus synchronization drugs in jennies and compare the efficiency of natural and synthetic prostaglandins in estrus synchronization.

Materials and Methods

Study area

This study was conducted in Debre Zeit located at about 45km southeast of Addis Ababa at 8^07^0N Latitude and 39^0E longitude at altitude of 1990m above sea level and situated in central Ethiopia. The climate is characterized by a bimodal rainfall with the short rainy season occurring from March to May preceded by a long dry season from October to February. The long rainy season occurs from June to September. The relative humidity is 52% and the annual rainfall of 866mm of which 84% falls during the long rainy. The mean maximum and minimum temperature ranges are 26^0C and 14^0C, respectively.

Study animals

A total of eighteen apparently healthy and none pregnant jennies whose ovarian follicular activities were previously determined (Sida/SAREC research project, 2007) were used in this study. The jennies were 6 - 14 years old, had a body weight of 120 - 140kg, and a body condition score of 3 to 4 (on a 0 - 5 scale). The jennies were housed in a closed barn during the night but were allowed to graze natural pasture during the day time. They were also supplemented with grass hay, and water was provided ad libitum on daily basis. All the jennies were regularly dewormed previously however, treatment was repeated for common internal and external parasites with Invermectin (Vermic® Centrovet, Chile) at a rate of 1ml/50kg orally one week before the start of the experiment.

Study design

Trial – I: synchronization with Lytalyse (natural prostaglandin)

The jennies were randomly assigned in to two groups: Lutalyse treatment (n=5) and Control (n=4). The jennies were given 1ml (equivalent to 5mg/ml) intramuscular injection of Lutalyse (Dinoprost tromethamine, Lutalyse®, USA) and the same treatment was repeated on Day 14. They were scanned using ultrasound (Mindray, veterinary digital ultrasonic imaging system, Hong Kong) prior

to the administration of Lutalyse and every other day starting on day 3 post injection for a total of 126 jenny days (JD) to determine the ovarian follicular activity. The animals were daily allowed to come in contact with jacks for about 20 minutes and were observed for manifestation of estrus. The number of follicles in each ovary, the diameter of the three largest follicles in each ovary (including the dominant/ preovulatory follicle), the number of days to estrus, length of estrus, and days to ovulation were recorded and used for later comparison of ovarian functions.

Trial – II: synchronization with Estrumate (synthetic prostaglandin)

The jennies were randomly assigned in to two groups: Estrumate treatment group (n=7) and control groups (n=2). The jennies were given 0.5ml (equivalent to 125 µg) intramuscular injection of Estruate (Cloprostenol®, Schering-Plough Animal Health Corp, Germany). They were similarly scanned using ultrasound (Mindray, Vet Digital Ultrasonic Imaging System, Hong Kong) prior to the administration of Estrumate and every other day starting on day 3 post injection for a total of 54 JD, to determine the ovarian follicular activity. The presence of estrus was observed daily. Ovarian function was studied similarly from the number of follicles in each ovary, the diameter of the three largest follicles in each ovary, the diameter of the dominant/ preovulatory follicle, days to estrus, length of estrus, days to ovulation and ovulation rate. Follicular sizes were determined using the internal electronic caliper of the ultrasound. Ovulation was confirmed after a sudden disappearance of a preovulatory follicle and ultrasonic detection of corpus luteum in the following days.

Data analysis

All data were stored in Microsoft Excel sheet and all computations and comparisons for each variable interaction was performed using SPSS for windows (SPSS version 15.0. 2006, Chicago, USA). The data was described and results were presented as mean (±Standard Deviation). Variables were compared using student t-test, ANOVA and Chi Square. P-value was held at 0.05 to determine significance of differences.

Results

Synchronization with Lutalyse

Except one jenny in the control group, no preovulatory follicles were present at the time of lutalyse injection in all animals. A corpus luteum was detected in 3 of the 5 treated jennies during scanning at the day of lutalyse treatment. All of the jennies exhibited signs of colic, straining, distress, frequent recumbency, sweating and urination shortly (10-15minutes) after injection. These signs receded soon and all the animals returned to normal state within 60 minutes of the injection. The mean (±SD) total number of follicles in both ovaries for all jennies was 12.6± 3.9 (range 6-21); while it was 12.9±3.8 follicles for Lutalyse treatment, and 12.2±4.2 follicles for Lutalyse control groups. Table 1 presents summary of the follicular data for both the treatment and control groups.

Follicular growth pattern with respect to the total number of follicles in both ovaries in the treatment and control groups were closely similar after the second injection (Figure 1). The mean (±SD) days to estrus, days to ovulation, and the rate of ovulation were 2.3 ± 0.5 days, 11.0 ± 1.2days, and 80%, respectively. Only 1 jenny ovulated after the first injection of lutalyse while 4 ovulated during the second injection. The average duration of estrus was 8days (range 6-12days).

The particular elements of behavioral manifestations of estrus in those jennies that showed estrus included lowering of the head with forward extension of the neck, jawing, backward depressing of the ears against the neck, raising the tail, frequent urination, positioning, vocalization, flehman reaction, mounting other jennies, and winking.

Synchronization with Estrumate

Jennies injected with estrumate did not show any clinical signs of side effects. The mean (±SD) total number of follicles in both ovaries for all jennies was 12.5 ±4.9 follicles (range 5-23), while it was 13.0±5 follicles for the treatment and 9.3±1.5 for the control groups. Follicular growth pattern showed a

Table 1: Size distribution of follicles in both ovaries of treatment and control jennies in Trial-I and II

Parameter of ovarian function	Lutalyse (126JD)		Estrumate (54JD)	
	Treatment	Control	Treatment	Control
Size of largest follicle on the date of treatment [mm]	15.1 (±1.1)	20.1 (±8.2)	18.8 (±5.7)	17.9 (±0.4)
Size of largest follicle in LOV [mm]	17.5 (±7.3)	19.3 (±9.2)	23.8 (±8.6)	19.7 (±5.7)
Size of largest follicle in ROV [mm]	17.1 (±7.8)	16.1 (±5.9)	17.2 (±7.9)	11.8 (±4.3)
Size of the preovulatory follicle [mm]	37.4 (±2.4)		39.5 (±2.7)	

JD= Jenny days; LOV= Left ovary; ROV= Right ovary

steady decrease in the total number of follicles in the treatment group (Figure 2) while it remained relatively unchanged for the control group. The mean (±SD) days to estrus, days to ovulation, diameter of the preovulatory follicle and the rate of ovulation in estrumate treatment group were 3.9± 0.7 days, 9.1 ± 1.6 days and 100%, respectively. The average length of estrus was 6.3 days (range 5 – 8 days). All the basic elements of behavioral estrus were present but their manifestation was weaker in spite of an apparent follicular activity evidenced by the presence of large follicles (>20mm).

There was a significant difference ($p<0.05$) in the mean diameter of the first largest follicles between the jennies of the two trials. Estrumate treated jennies had the largest follicular diameter in both ovaries (23.8±8.6mm for left and 17.2±7.9mm for right ovaries), compared with the jennies treated with Lutalyse (17.5±7.3mm for left and 17.1±7.8 for the right ovaries). However, there was no significant difference both in total number of follicles and diameter of the preovulatory follicle between the trials.

In the Trial-I, 3 out 5 jennies (60%) showed estrus signs on Day 2 post Lutalyse injection while in Trial-II, 4 out of 7 jennies (57%) showed estrus on Day 4 of Estrumate injection. The days to estrus was significantly shorter ($p<0.05$) with Lutalyse treatment while the days to ovulation was significantly shorter ($p<0.05$) with Estrumate treatment. Preovulatory follicles were relatively larger in Estrumate treated groups (39.45 ± 2.7mm) than Lutalyse treated jennies (37.35 ± 2.4mm) but the difference was not statistically significant. The maximum diameter of the preovulatory follicle (41mm) was found in jennies treated with Estrumate.

Discussion

The dose of both lutalyse and Estrumate were based of the dose rates recommended for mares (Samper, *et al* 2006). Thus the present study showed that dose rates recommended for mare are also working for jennies. However, clinical side effects were more pronounced with lutalyse requiring further studies to determine the optimal dose rate for jennies to induce luteolysis. Estrus manifestations and follicular activities with regards to the mean number of follicles, the diameter of the preovulatory follicles and length of estrus found in both trials are closely similar to previous studies for donkeys (Henry *et al* 1991; Meira *et al* 1995; Henry *et al,* 1998; Blanchard, *et al,* 1999; Lemma *et al,* 2006).

The variations in the number of ovarian follicles visible at different stages of the reproductive cycle and the maximum size of a preovulatory follicle in jennies have also been previously discussed (Meira *et al* 1995; Lemma *et al,* 2006). Carluccio and colleagues (2006; 2008) similarly reported a mean preovulatory follicular size of 39± 0.27mm in jennies following $PGF_{2\alpha}$ treatment.

The length of estrus, duration from treatment to estrus found with lutalyse treatment of this study (8 days and 2.3±0.5 days) are in close agreement to previous reports (Henry *et al,* 1998; Carluccio *et al* 2006; 2008) but is shorter than the 4.4±1.6 days reported by Blanchard *et al,* (1999). On the other hand, the duration from treatment

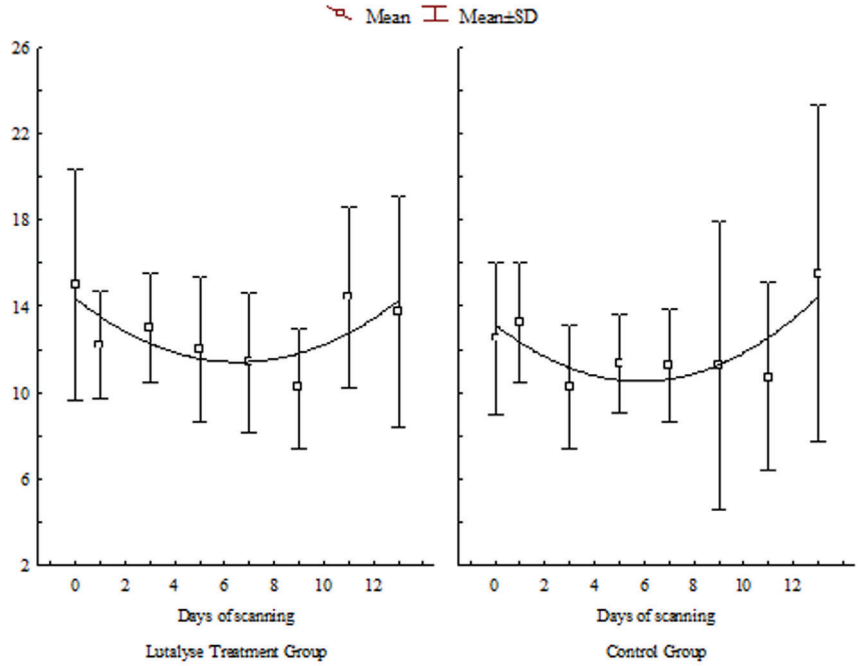

Figure 1: Follicular development pattern in the treatment and control jennies (n=9) from the days of the second treatment of Lutalyse (Day 0)

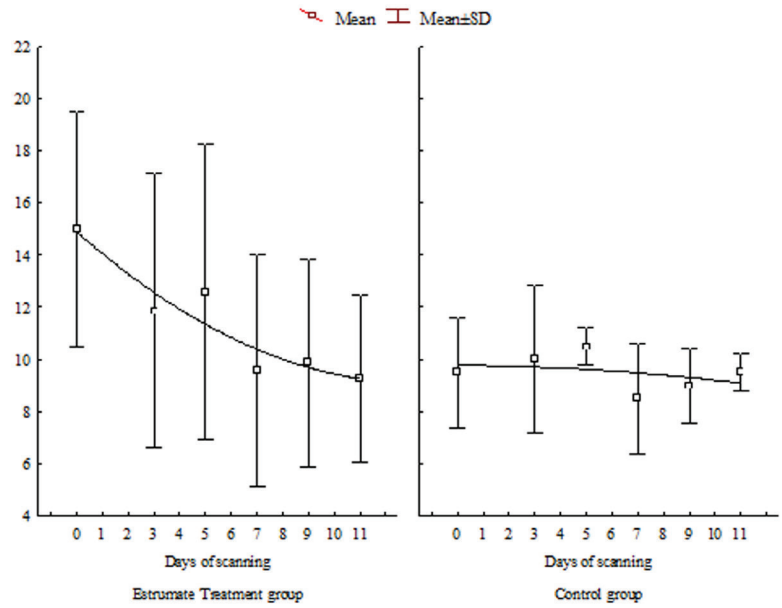

Figure 2: Follicular development pattern with respect to total number of follicles in Estrumate treated and control jennies (n=9)

to ovulation was similar to the finding by the later authors. This study demonstrates that donkeys are responsive to different doses and preparations of prostaglandin administered at any time following ovulation. Different studies indicated the use of natural prostaglandin to be effective in synchronizing estrus in mares starting on day 6 post ovulation through day 18 of the cycle. After treatment, the mares were known to return to estrus in 4-5days, and ovulate 10-12 days post injection (Bearden *et al,* 2004; Samper, 2008). However, large dose

normally recommended for mares is still of concern due to the side effects. No reports so far exist on the use of estrumate in jennies for estrus synchronization but results of the current study are generally in close agreement with reports for mares (Samper, 2008).

The length of estrus, duration from treatment to estrus found with lutalyse treatment of this study (8 days and 2.3±0.5 days) are in close agreement to previous reports (Henry *et al*, 1998; Carluccio et al 2006; 2008) but is shorter than the 4.4±1.6 days reported by Blanchard *et al,* (1999). On the other hand, the duration from treatment to ovulation was similar to the finding by the later authors. This study demonstrates that donkeys are responsive to different doses and preparations of prostaglandin administered at different times following ovulation. Different studies indicated the use of natural prostaglandin to be effective in synchronizing estrus in mares starting on day 6 post ovulation through day 18 of the cycle. After treatment, the mares were known to return to estrus in 4-5days, and ovulate 10-12 days post injection (Bearden *et al,* 2004; Samper, 2008). However, large dose normally recommended for mares is still of concern due to the side effects. No reports so far exist on the use of estrumate in tropical jennies for estrus synchronization but results of the current study are generally in close agreement with reports for mares (Samper, 2008).

Conclusion

From the present study it is concluded that estrus can be synchronized using both natural and synthetic prostaglandins at a dose rate recommended for mares. However, synchronization with estrumate treatment produced a relatively better response than lutalyse treatment. Although days to estrus were shorter and estrus manifestations were overt during lutalyse treatment, follicular activity was better with estrumate treatment evidenced by the appearance of larger follicles and higher incidence of ovulation. A further study on the optimal doses of lutalyse for jennies is recommended.

Impact

In many African countries, the contribution of donkeys in the agricultural sector is crucial. Donkeys in most instances were considered as small horse regarding reproduction while they are not. Detection of estrus in group of randomly cycling females is time consuming, and laborious. Synchronization of estrus and ovulation time solves such problem so that the females would be in estrus within a predictable time. Particularly, synchronization of estrus, little known for tropical jennies, is highly useful in the selection of jennies, and jacks with useful traits to breed in a more controlled manner and contribute to better reproductive management

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SEROLOGICAL SURVEY OF MAEDI-VISNA VIRUS INFECTION IN HIGHLAND SHEEP AT RANCHES AND SMALLHOLDER FARMS IN EASTERN AMHARA REGION, ETHIOPIA

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Abstract

A cross-sectional study was conducted to determine the prevalence and associations with potential risk factors of Maedi-visna virus infection in the Ethiopian highland sheep of the eastern Amhara region. A total of 2417 sheep were examined between December 2005 and March 2006. Of these, 926 sheep were from semi-intensive production system (ranches) composed mainly of Awassi and indigenous Menz sheep breeds while 1491 sheep were from extensive system (smallholder farms) with indigenous breeds being predominant. Indirect-Enzyme Linked Immunosorbent Assay and Agar Gel Immunodiffusion tests were employed in parallel to determine the presence of antibodies against maedi-visna virus infection. The overall individual animal-and flock-level prevalence was 15.6% (95%CI: 14.1-17.1) and 25.9% (95%CI: 20.8-31.5), respectively. Maedi-visna seroprevalence was higher in sheep at ranches with prevalence of 30% and 86.2% at individual- and flock-level. A Prevalence of 6.6% and 18.8% was found in smallholder farms at individual- and flock-level, respectively. The prevalence difference was highly significant ($P < 0.001$) between the sheep production systems. Breed, flock size, age, sex, and husbandry practices were significantly associated with maedi-visna seropositivity. Higher risk to infection was found in Awassi breed and their crosses than indigenous sheep. Breed management systems, but not breed caused susceptibility variation. The husbandry and management systems, old and large flock sizes in ranches were found important risk factors associated with higher rate of infection. Sheep breeding ranches could serve as a source of Maedi-visna virus infection to smallholder farms along with the distribution of rams and effective control measures have to be implemented through annual testing and culling of seroreactors and raising lambs artificially in isolation. Screening tests aiming at culling seropositive animals should be carried out during introduction of new flocks and before distribution of rams to smallholder farms.

Keywords: AGID, ELISA; Maedi-visna virus; seroprevalence; smallholder farm; ranch

ETUDE SÉROLOGIQUE DE L'INFECTION CAUSÉE PAR LE VIRUS MAEDI-VISNA CHEZ LES MOUTONS DES HAUTS-PLATEAUX DANS LES FERMES ET LES PETITES EXPLOITATIONS DE LA RÉGION AMHARA ORIENTALE ENETHIOPIE

Résumé

Une étude transversale a été menée afin de déterminer la prévalence et les relations avec les facteurs de risque potentiels de l'infection par le virus Maedi-visna chez le mouton des hauts plateaux éthiopiens de la région Amhara orientale. Au total, 2.417 moutons ont été examinés entre décembre 2005 et mars 2006. De cet ensemble, 926 moutons provenaient du système de production semi-intensif (ranch) composé principalement de races ovines Awassi et de Menz indigènes, tandis que 1.491 moutons provenaient d'un système extensif (petites exploitations) avec une prédominance de races indigènes. L'épreuve immuno-enzymatique indirecte et l'épreuve d'immunodiffusion en gélose ont été utilisées en parallèle pour déterminer la présence d'anticorps du virus Maedi-visna. Le taux de prévalence global aux niveaux de l'animal individuel et du troupeau était respectivement de 15,6% (95%CI: 14,1 – 17,1) et de 25,9% (95%CI: - 20,8 - 31,5). La séroprévalence était plus élevée chez les ovins des ranchs où elle était de 30% et de 86,2% respectivement au niveau individuel et au niveau du troupeau. Un taux de prévalence de 6,6% et de 18,8% a été noté respectivement dans les petites exploitations au niveau individuel et au niveau du troupeau. La différence au niveau de la prévalence était très significative ($P < 0,001$) entre les systèmes de production ovine. La race, la taille du troupeau, l'âge, le sexe et le système d'élevage étaient significativement associés à la séropositivité au maedi-visna. Un risque d'infection plus élevé a été identifié

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chez les moutons de la race Awassi et ses croisés par rapport aux moutons indigènes. Les systèmes de gestion des races, mais pas les races elles-mêmes,ont causé une variation de susceptibilité. Les systèmes d'élevage et de gestion, les tailles de troupeaux anciens et grands dans les ranchs ont été identifiés comme d'importants facteurs de risque associés à un taux d'infection plus élevé. Les ranchs d'élevage de moutons pourraient servir de sources d'infection par le virus Maedi-visna pour les petites exploitations de même que la distribution de béliers.. Des tests de dépistage visant l'abattage d'animaux séropositifs devraient être effectués lors de l'introduction de nouveaux troupeaux et avant la distribution de béliers aux petites exploitations.

Mots-clés: AGID, ELISA, Virus maedi-visna, Séroprévalence ,moutons Petite exploitation, Ranch

Introduction

Maedi-visna (MV) is a chronic disease of sheep produced by a member of the genus Lentivirus, in the family Retroviridae, which causes life-long systemic and progressive infection with slow development of disease, beginning in subclinical form and leading to severe disability and death. The main target cells of maedi-visna virus (MVV) are of the monocyte/ macrophage lineage and it does not infect T-lymphocytes or cause immune suppression. Lentiviruses of sheep are known by different names such as maedi-visna virus, ovine progressive pneumonia virus and ovine lentivirus (Torsteinsdottir *et al.,* 2007).

Although most infections are subclinical, a minority of animals develop progressive, untreatable disease syndromes including dyspnea (maedi) or neurologic signs (visna). The disease is mainly transmitted from infected ewes to their lambs; by the ingestion of virus-containing colostrum or milk early in life. The virus can also be spread during close contact, probably by the respiratory route (Blacklaws *et al.,* 2004). Most infections are asymptomatic, but once clinical signs appear, the disease is progressive and usually fatal. Clinical disease is chronic and characterized by mononuclear cells infiltration of target organs including the lungs, mammary glands, joints and central nervous system. At the start of the infection process the manifestations are imprecise and very difficult to detect. As the disease progresses; cachexia, dyspnea, mammary hardening, paralysis and lameness develop (Cutlip *et al.,* 1992).

Maedi-visna virus persists and replicates in the presence of active humoral and cellular immune responses and causes immune-mediated lesions in several organs and this unusual relationship with the host makes diagnosis and control difficult and expensive (Callado *et al.,* 2001). Vaccination attempts have not only induced sterile immunity but have occasionally caused increased viremia and more severe disease (Petursson *et al.,* 2005). Diagnosis of MVV is primarily based on the presence of antibodies usually detected by Agar Gel Immunodiffusion (AGID) or Enzyme Linked Immunosorbent Assays (ELISA) (de Andres *et al.,* 2005; Lacerenza *et al.,* 2006). As no effective vaccine is available, most often employed schemes to prevent spread of MVV are based on segregation and culling of positive animals associated with management practices, especially the offspring (Callado *et al.,* 2001).

Maedi-visna has been found in most sheep-raising countries other than Australia and New Zealand. It has been reported from most of continental Europe, the United Kingdom, Canada, the United States, Kenya, South Africa, Israel, India and the southern regions of the former U.S.S.R (Clements and Zink, 1996). When MVV is introduced into a new area, the mortality rate may reach 20-30%. The mortality rate is low in regions where MV is endemic; annual losses rarely exceed 5% in a flock, even when nearly 100% of the flock is infected (Peterhans *et al.,* 2004). The emergence of MVV in Ethiopia was serologically detected for the first time in imported Merino sheep in 1986 and followed by the report of 3.7% prevalence around Debre Berhan in indigenous sheep (Ayelet *et al.,* 2001). Tibbo *et al.* (2001) and Woldemeskel *et al.* (2002) have also reported MV in Awasssi sheep at Debre Berhan and Amed Guya breeding ranches. Despite these few reports, information was limited on the magnitude of the disease in Ethiopian highland sheep. Hence this study was undertaken with the objectives of determining

the magnitude of the disease and identifying associated risk factors for the occurrence of MVV in adult sheep.

Materials and Methods

Study area

The study was conducted in eastern Amhara region of Ethiopia. The study area included three Administrative zones of Amhara National Regional State namely; North Shewa, North and South Wollo. These zones are the major areas where sheep husbandry is widely practiced as a major component of livestock production and used as main source of meat and wool. Both extensive and semi-intensive sheep production management is practiced in the highlands of these areas. The indigenous sheep are mainly kept extensively by smallholder farmers while Awassi breed and crosses are managed at breeding and multiplication ranches mainly in North Shewa administrative zone. The altitude of the study area ranges from 2500 to 3100 meters above sea level with annual rainfall ranging between 500 and 1000mm with bimodal rainfall in pattern. Average monthly minimum air temperature ranges 2.4^0C in November to 8.5^0C in August where as the average monthly maximum air temperature ranges from 18.3^0C to 27.3^0C in June.

Study animals

The study was conducted on sheep owned by smallholder farmers and government. The study sheep comprised of both indigenous breeds and imported Awassi sheep. The indigenous sheep included the Menz (N. Shewa) and Tikure (Wollo) sheep. The Awassi sheep were introduced in 1985 and 1994 from Israel for cross breeding with indigenous sheep mainly with Menz sheep for mutton and wool production. The Awassi sheep are kept in large flocks under semi-intensive management at ranches and their crosses had frequently been distributed to smallholder farmers in Amhara region and other part of the country through the animal extension service.

Study design

A cross-sectional study was conducted during the period from December 2005 to March 2006 using serological procedures. The serological survey was carried out with the intention of determining individual animal- and flock-level prevalence. Blood sampling was performed from selected individual sheep of both sexes, aged 1year and over. The blood was allowed to clot and the sera were separated by centrifugation and stored at -20°C until tested. Information of each sheep sampled were obtained including; its location, flock size, sex, age and health status.

Sample size determination

Two types of sheep production systems were classified as extensive and semi-intensive. The extensive system consists of mostly indigenous sheep and in some pockets of cross-breeds. The semi-intensive production system contains mainly Awassi and Menz sheep breeds and depends on partial feed supplementation and is allowed to graze in flock in enclosed ranches.

The true representatives of the study population were selected by combination of stratified and systematic sampling methods. The sample size was determined at 7% estimated prevalence of MVV antibodies and with a desired absolute precision of 2%. The estimated prevalence was based on a previous report of MV (Ayelet *et al.,* 2001). Sample size calculation was performed based on Thrusfield (2005) using the formula for random sampling: $n = t_x^2 * P(1-P)/L^2$; Where, n = sample size, tx = student t-value (1.96 at 95%), P = estimated prevalence of MV, L = desired absolute precision).

The study population was stratified into two strata based on the management types; sheep flocks in ranches and smallholder farms, which could influence the prevalence of MV antibodies to be estimated. Stratification with a variable sampling fraction was used and the proportional allocation was 20% for ranch sheep flocks and 0.20% for flocks of smallholder farms. A total of 2417 adult animals in 274 flocks were sampled for the study.

Data and serum collection

Data and sera were collected from 2417 adult sheep. Data collected at the time

of blood sampling included the farm type, age of animal, sex, breed description of the animal, and vial number. Whole blood was collected aseptically from the jugular vein of each sample animal using 10 ml non-heparinized vacutainer tubes. Sera were extracted and held under refrigeration until processed.

Serological testing

All sera were processed and tested in parallel by indirect-ELISA and AGID protocols using readily available kits containing all necessary antigens, positive and negative control and reagents. Both kits used were products of Pourquier Institute, France. The test procedures recommended by the manufacturer were strictly followed in both tests for MVV antibody verification.

The ELISA method used for the detection of MV antibodies was an indirect-ELISA based on the use of an immunogenic peptide of a trans-membrane protein (TM, ENV gene) and on the other hand, on the use of the recombinant P28 protein, which enters into the composition of the viral capsid (gag gene). The use of the conserved viral capsid protein (P28) allows the serological detection of a very wide spectrum of serological variants; the use of the trans-membrane protein allows a premature detection of infection and improves the sensibility of the test.

The AGID test was used to detect antibodies to the MV viral envelop glycoprotein (gp135) using the agar gel immuno-diffusion technique. The test uses Ouchterlony method (also called double immuno-diffusin method) of precipitation on agar-gel.

Data management and analyses

Data obtained from both serological tests were stored in Microsoft access database (Microsoft Corp.). These data were analyzed by descriptive statistics, univariable and multivariable regression using the STATA 11.0 statistical package (StataCorp, 2004). Serum reacted positive with either of the serological tests was considered infected with MV virus. Flock having at least one seropositive sheep was considered positive. Logistic regression fitted model used for the analysis of the effect of exposure factors on seropositivity of MV antibodies. Variables with $P<0.2$ were identified as risk factors for inclusion in multivariable regression model. They were selected for inclusion in the final model on the basis of P-value <0.05 in a backward selection procedure using STATA software.

Results

Seroprevalence of Maedi-visna in sheep

A total of 2417 sheep sera (1491 in smallholder farms and 926 in ranches) were examined from eastern Amhara region for the presence of serum antibodies against MVV infection. The sera were tested using established ELISA and AGID tests. The overall apparent seroprevalence of MV in eastern Amhara region was 15.6% (376/2417). The individual-level prevalence was 6.6% (95%CI: 5.4-7.9) in smallholder farms and 30% (95%CI: 27.1-33.1) in breeding sheep at ranches (Table 1). The MV prevalence distribution had varied among the districts, higher in Gera-keya (15.4%) and Basona-worana (6.9%) in North Shewa followed by Wadilla (4.5%) in North Wollo administrative zone. In ranches, the prevalence was much higher in Sheno (87.4%) and lower at Guguftu (3.1%). The prevalence variation was significant ($p=0.001$) among the districts and ranches and between the sheep production systems.

The mean flock seroprevalence with at least one seropositive sheep was 25.9% (71/274). The flock-level prevalence distribution in production system was 18.8% (95% CI: 14.1-24.2) in smallholder farms and 86.2% (95%CI: 68.3-96.1) in ranches (Table 1). In smallholder farms, the flock seropositivity varied considerably among the districts and it was higher in Gera-Keya (41.7%) followed by Basona-worana (33.3%) while in ranches all flocks (100%) in Sheno and Amed-guya had at least one sheep seropositive.

Univariable and multivariable logistic regression analyses of risk factors for Maedi-visna virus infection in sheep

The differences between MV seroprevalence in sheep per each risk factor categories as well as their associations are summarized in Table 2. During the statistical

analyses of all risk factors, the first level of each independent variable was used as a reference category. The result indicated that seropositivity to MV antibodies in sheep at ranches was significantly (P=0.001) higher than in the smallholder farms. There were also significant (P=0.001) differences among the breeds. Awassi breed demonstrated higher prevalence (77.6%) compared to Awassi-indigenous crossbreds (60.2%) and indigenous sheep (16%). Univariable logistic regression model showed that sheep with ages of above 6 years and large flocks demonstrated higher prevalence of 47.6% and 98.6%, respectively and the difference was highly significant (P=0.001). Male sheep demonstrated higher seroprevalence. However, the ratio of male to female sheep tested was 1:7.

The multivariable logistic regression model (Table 2) adjusted for assumed risk factors showed as production system, sex, age, breed and flock size were significantly(p=0.001) associated with seropositivity of MV in sheep.

Comparison of ELISA and AGID tests

A summary of the ELISA and AGID test results for the sera at the individual animal- and flock-level used for the calculation of sensitivity and specificity of the tests is presented in Table 3. Values calculated at the individual-level for the sensitivity and specificity were 91.8%, (95% CI: 89.6-94.3%) and 97.5% (95% CI: 95.4-99.8%), respectively. Similarly, the sensitivity and specificity of the tests at herd-level were 93.8% (95% CI: 91.7-95.6%) and 97.8% (95% CI: 95.7-100%), respectively.

Test agreement between ELISA and AGID based on the crude agreement (concordance) and the agreement beyond chance (Kappa statistics) were also performed on sheep sera samples from both production systems; smallholder farms and ranches (Table

Table 1: Individual- and flock-level Maedi-visna seroprevalence in sheep managed under extensive and semi-intensive production system of eastern Amhara region, Ethiopia.

Production system	Zone	District	Individual seroprevalence			Flock seroprevalence		
			N_i	Preval (%)	95% CI	N_b	Preval (%)	95% CI
Extensive (Smallholder farm)	N.Shewa	Gera-keya	364	15.4	11.8-19.5	21	33.3	14.6-56.9
		Basona-worana	202	6.9	3.8-11.4	36	41.7	25.5-59.2
	S.Wollo	Dessie-zuria	240	2.5	0.9-5.4	38	10.5	2.9-24.8
		Legambo	181	2.2	0.6-5.6	77	7.8	2.9-16.2
	N.Wollo	Meket	259	2.7	1.1-5.5	43	16.3	6.8-30.7
		Wadilla	245	4.5	2.3-7.9	30	23.3	9.9-42.3
	total		1491	6.6	5.4-7.9	245	18.8	14.1-24.2
Semi-intensive (Ranch)	N.Shewa	Debre-berhan	335	20.0	15.9-24.7	12	75.0	42.8-94.5
		Amed-guya	375	20.5	16.6-24.9	7	100	59.0-100
		Sheno	151	87.4	81.1-92.3	7	100	59.0-100
	S.Wollo	Guguftu	65	3.1	0.4-10.7	3	66.7	9.4-99.2
	total		926	30.0	27.1-33.1	29	86.2	68.3-96.1
	Overall		2417	15.6	14.1-17.1	274	25.9	20.8 - 31.5

N_i: total individual animals tested; N_h: total herds sampled; C_i: confidence interval

Table 2: Summary results of the univariable and multivariable logistic regression analyses (LR) of risk factors with dependent Maedi-visna seropositivity in sheep in eastern Amhara region

Risk factors	Category levels	N*	Preval (%)	Univariable LR analysis results			Multivariable LR analysis results		
				P-value	OR	95%CI	P-value	OR	95%CI
Production system	Smallholder farm	1491	6.6	0.001	6.1	4.8-7.8	0.01	2.3	1.5-3.5
	Ranch	926	30.0						
Sex	Female	2118	14.4	0.001	1.9	1.4-2.5	0.001	3.3	2.2-5.2
	Male	299	24.1						
Age	1-3	1347	10.2	0.001	-	-			
	3-6	947	19.0	0.001	2.1	1.6-2.6			
	>6	123	47.2	0.001	7.8	5.3-11.6	0.001	4.0	3.2-5.1
Breed	Indigenous	658	16.0	0.001	-	-			
	Awassi x indigen.	201	60.2	0.001	5.8	4.5 7.6			
	Awassi	67	77.6	0.001	32.8	18.1-59.5	0.001	3.4	2.5-4.5
Flock size*	Small	179	14.0	0.001	-	-			
	Medium	66	42.4	0.001	4.6	1.5-7.8			
	Large	70	98.6	0.001	9.6	6.4-14.3	0.001	3.0	2.3-4.1

N*: number of animals tested; Flock size*: small (3-10); medium (11-20); large (>20)

Table 3: Summary of ELISA and AGID test results of Maedi-visna virus infected sera in individual- and flock-level of sheep in eastern Amhara region.

	Individual animal level		Total	Flock level		Total
	AGID(+ve)	AGID(-ve)		AGID(+ve)	AGID(-ve)	
ELISA(+ve)	324	52	376	45	5	50
ELISA(-ve)	29	2012	2041	3	221	224
Total	**353**	**2064**	**2417**	**48**	**226**	**274**

Table 4: Concordance and kappa statistics of ELISA and AGID tests on sheep sera samples

Production system	N*	Serological Assay		Concordance (%)	95%CI	Kappa Value	95%CI	Agreement
		ELISA	AGID					
Smallholder farms	1491	98	75	97.4	95.9-98.4	0.76	0.00-0.94	Substantial
Ranches	926	278	278	95.4	91.4-96.9	0.89	0.00-0.96	Almost perfect
Total	**2417**	**376**	**353**	**96.6**	**94.7-98.2**	**0.87**	**0.83-0.91**	**Almost perfect**

N*: number of sera samples tested; CI: confidence interval

4). The mean concordance and kappa value for overall sera samples regardless of the origin, was found to be 96.6% (95%CI: 94.7-98.2) and 0.87 (95%CI: 0.83-0.91), respectively. The statistical agreement between the ELISA and AGID tests was almost perfect.

Discussion

Seroprevalences of MV have been established at different times from various countries (Cutlip *et al.,* 1992; Clements and

Zink, 1996; Peterhans *et al.,* 2004). There was no adequate information however on the status of the disease in Ethiopia. An overall seroprevalence of 15.6% was found in our study area indicating the occurrence and wide distribution of MV virus infection in both sheep production systems in eastern Amhara region. The rates of infection vary greatly from one country to another, within a country and production systems (Brodie et al., 1998). Comparison of seroprevalence in the different production systems indicated that the occurrence of the disease, both at individual- and flock-level was lower in smallholder farms than that of the ranches. There was significant variation (P=0.001) between the production systems. This variation difference in production system is in agreement with previous reports. Ayelet et al. (2001) has reported lower prevalence (3.7%) of MV in village flocks and relatively higher (7%) in sheep on-station at Debre-birhan, N. Shewa. Woldemeskel et al. (2002) also reported higher seroprevalence (74%) of MV in clinically morbid sheep at ranches. The seroprevalence difference between the ranches and smallholder farms was probably associated with the husbandry and management practices. The large flock size, confining animals for longer hours during cold seasons, mixing different breeds and age groups, keeping high proportion of older animals in ranches have been incriminated to contribute for the disease occurrence and transmission in higher rates. Baumgartner *et al.* (1990) have suggested unfavorable housing conditions such as insufficient room, bad climatic conditions and crowding behavior in sheep promote a high incidence of the disease.

The individual-level seroprevalence difference among sheep ranches was significantly (P=0.001) higher in Sheno (87.4%) than other ranches. The lower prevalence in Debre-berhan and Amed-guya was due to mass culling of the old flocks and about 80% of the flocks were replaced with new flocks of indigenous sheep a year before this study while the old sheep population in Sheno has been kept undisturbed. Cutlip et al. (1992) also reported increased prevalence with age from 4% in less than 1 year to 34% in 4 years period. The prevalence difference among the districts was significant (p=0.001). The higher prevalence in Gera-keya (15.4%) and Basona-worana (6.9%) districts of North Shewa administrative zone is the reflection of frequent contact and/or higher exposure of healthy village sheep to infected animals in ranches at Debre-berhan, Amed-guya and Sheno which are located in these districts. Smallhoder farmers around these ranches had frequent access to purchase rams and culled sheep. Besides loose fences of ranches allowed animal contacts between ranch and adjacent farms. This reality holds true for higher risk and pressure of MVV infection to flocks in adjacent villages to ranches.

The seroprevalence finding in North Wollo (3.6%) and South Wollo (2.4%) administrative zones and their respective districts is quiet interesting. These zones are geographically located far from severely affected sheep ranches and it is suggested that the disease might have been spread along with the distribution of Awassi crossbred rams as we investigated seroreactor rams in the villages obtained from ranches a year ago.

Univariable and multivariable logistic regression analysis of the risk factors indicated that production system, breed, flock size, age, and sex were highly associated with MV seropositivity. Stocking densities are important potential determinants for MV virus transmission (Peterhans *et al.,* 2004; Blacklaws *et al.,* 2004). This concept coincides with the current study that the seroprevalence of MV among three categorized flock sizes showed significant variations with higher prevalence recorded in the large flock size. The odds ratio (OR) indicated that the large flock size was 9.6 times more likely to be reactors than animals in small flock size indicating greater exposure of animals to lactogenic and horizontal transmission. This agrees with previous studies in Canada and USA that they have reported increased prevalence with increasing flock size ranged from 19% to 97% (Constable *et al.,* 1996; Kozaczynska *et al.,* 2002).

An attempt was also made to compare the seropositivity rates between Awassi and indigenous breeds of sheep. Higher seroprevalence with significant difference (P=0.001) was revealed in Awassi breeds. The Awassi sheep were more likely at risk (OR,

32.8) to acquire MV virus infection than the indigenous sheep and crossbreds. Snowder *et al.* (1990) and Schaller *et al.* (2000) have also reported a prevalence difference among breeds. However, complete breed associated resistance has not been demonstrated.

A significant seroprevalence difference (P=0.001) among age groups was observed. The prevalence increased starting from 4 year of age and could probably be explained by the longer exposure of animals to horizontal transmission and the delay of seroconversion following infection. The older animals (>6years) were more likely at risk (OR, 7.8) of getting MV virus than lower age groups. This was consistent with the works of Cutlip *et al.* (1992) and Snowder *et al.* (1990) who reported 4% and 11% at one year of age and 34% and 93% at above 4 years of age, respectively

In this study, male sheep demonstrated higher prevalence (P = 0.001) than female animals. Similarly, Simard and Morley (1991) reported an association between sex and seropositivity. However, there is no other reported evidence of a difference in susceptibility to MVV infection between the sexes. The difference in this study could be related to the sheep management where large proportion of young female was lately introduced to the ranches.

Comparison of serological tests (ELISA and AGID) for sera samples demonstrated a crude agreement of 96.6% and kappa value of 0.87, which was almost perfect agreement. The ELISA kit used in this study was 6.1% more sensitive than the AGID test. Although AGID has been widely used as a prescribed test for international sheep trade (OIE, 1996), it has limitations and various ELISA protocols have been described for routine diagnosis. Different works have been done comparing different ELISA formats for MVV with the conventional AGID and the ELISA test was found to be more sensitive than the AGID test (Kozaczynska et al., 2002; Saman et al., 1999). However, accurate serological diagnostics should be based on the combination of the tests.

In conclusion, the results of the present study revealed that MV is widely distributed in Ethiopian highland sheep in eastern Amhara region. The rate was higher in semi-intensive production system (sheep breeding ranches). However, the apparently higher MV prevalence in imported Awassi sheep as compared to the indigenous breeds is attributed to the management system by which Awassi is reared. The presence of higher positive seroreactors among the Awassi sheep was mutually exclusive with the strategy of the regional government of providing Awassi crossbred rams to smallholder farmers to up-grade the genetic potential of indigenous sheep for mutton and wool production. Hence, the finding of positive serological reactors does not only suggest the occurrence of the disease in sheep population of the study area, but also indicates the presence of foci of infection that could serve as source of infection for the spread of the disease into unaffected animals around and elsewhere in the sheep producing areas. The husbandry and management systems and flock sizes were found important risk factors associated with MV seroreactors. Sheep breeding ranches were incriminated as a home-base for MVV infection and effective control measures should be implemented; i, through annual or semi-annual testing and culling of all seroreactor ewes and their progeny; ii. by removal of lambs at birth before taking colostrums and raising them artificially in isolation either on pasteurized milk or milk substitute. Simultaneously with regional government, screening test should be carried out during introduction of new flocks and before distribution of Awassi crossbred rams particularly from ranches to smallholder farms.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the work.

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VAGINAL CYTOLOGY PATTERN AND BIRTH FEATURES OF FEMALE WISTAR RATS TREATED WITH GRADED DOSES OF ETHANOLIC EXTRACT OF SPONDIAS MOMBIN

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Abstract

Twenty-five virgin female wistar rats weighing between 120g and 200g and divided into five groups of five rats per group were treated with graded oral dosages of ethanolic extract of Spondias mombin. Dosages served were 800mg/kg, 600mg/kg, 400mg/kg and 200mg/kg for groups A, B, C, D respectively and distilled water for group E which served as control. Five untreated proven male were used for copulation, one per group. Vaginal cytology was done daily for two weeks before treatment with extract to establish an oestrous cycle pattern repeated daily for another two weeks simultaneously with extract administration. Mating, pregnancy diagnosis and determination of birth parameters followed immediately after the end of extract treatment. Vaginal smears prior treatment and during treatment contained epithelial cells consistent with the different phases of estrus in the wistar rat and provided a cyclical pattern indicating that Spondias mombin had no negative effect on the estrous cycle of the wistar rat. Pregnancy and birth rates were favoured with groups A (800mg/kg) and E (control). Average litter sizes in all the groups were not significantly different . The average live birth weight of the neonates measured was observed to be highest for Group A with average live birth weight of 6.27g, followed by Groups B and the control with average live birth weight of 5.83g and 5.50g. The Groups C and D had lower average live birth weights of 4.01g and 4.74g respectively. The group A average weight was significantly high compared with other treatment groups and control ($p<0.05$). It was concluded that ethanolic extract of Spondia Mombin at 800mg/kg fed orally before copulation had no anti fertility effect on female wistar rat instead appeared to potentiate gestation parameters but same could not be said for dosages as low as 200mg/kg.

Key words : Antifertility, copulation, dosage, estrous cyle, litter size, Gestation.

PROFIL CYTOLOGIQUE VAGINAL ET CHARACTERISQUES DES MISE-BAS CHEZ LES RATS WISTAR FEMELLES TRAITÉES AVEC DES DOSES PROGRESSIVES DE L'EXTRAIT ÉTHANOLIQUE DE SPONDIAS MOMBIN

Résumé

Vingt-cinq rats Wistar femelles vierges pesant entre 120g et 200g et réparties en cinq groupes de cinq rats par groupe ont été traitées avec des doses orales progressives de l'extrait éthanolique de Spondiasmombin. Les doses servies étaient 800mg/kg, 600mg/kg, 400mg/kg et 200mg/kg respectivement pour les groupes A, B, C, D, et de l'eau distillée pour le groupe E servant de témoin. Cinq mâles non traités ont été utilisés pour la copulation, un par groupe. Une cytologie vaginale a été faite tous les jours pendant deux semaines avant le traitement avec l'extrait, afin d'établir un modèle de cycle œstral répété quotidiennement pendant deux autres semaines en même temps que l'administration de l'extrait. L'accouplement, le diagnostic de grossesse et la détermination des paramètres de mise-bas ont suivi immédiatement après la fin du traitement à base de l'extrait. Des frottis vaginaux avant et pendant le traitement contenaient des cellules épithéliales cohérentes avec les différentes phases de l'œstrus du rat Wistar et ont fourni un modèle cyclique indiquant que Spondiasmombin n'avait aucun effet négatif

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sur le cycle œstral du rat Wistar. Les taux de grossesse et de naissance étaient plus élevés chez les groupes A (800mg/kg) et E (témoin). Les tailles moyennes des portées dans tous les groupes n'étaient pas significativement différentes. Le poids moyen le plus élevé à la naissance des nouveau-nés vivants mesurés a été observée chez le groupe A qui était de 6.27g, suivi du groupe B et du groupe témoin, respectivement avec un poids moyen à la naissance de 5,83g et de 5,50g. Les groupes C et D avaient des poids moyens plus faibles, respectivement 4.01g et 4.74g.Le poids moyen chez le groupe A était significativement élevé par rapport aux autres groupes de traitement et au groupe témoin ($p < 0,05$). Il a été conclu que l'extrait éthanolique de Spondiasmombin à 800mg/kg administré oralement avant la copulation n'avait aucun effet anti-fertilité sur le rat Wistar femelle, au contraire il a semblé améliorer les paramètres de gestation, mais on ne peut pas en dire autant des doses aussi faibles que 200mg/kg.

Mots-clés : Anti-fertilité, Copulation, Dose, Cycleœstral, Taille des portées, Gestation.

Introduction

The reproductive health of female animals is essential in productivity. This informs the need to constantly protect the integrity of the different elements that combine to ensure non occurrence of reproductive failure.

Naturally occurring medicinal preparation from medicinal plants are utilised as therapy for various disease condition being preferred to synthesised drugs for the obvious reason of cost effectiveness and less exposure to chemical toxicity. Spondias. mombin is one of such medicinal plants used by many. It is among the forages given to domestic animals in Nigeria. The young leaves are also cooked as green vegetables by local folks (Ayoka *et al,* 2008). It is a fruitiferous tree that thrives in the rainforest and coastal areas of Africa. It is known by various names in Nigeria (Ibo: Ichikara, Hausa: Tsardarmasar, Yoruba: Iyeye). The therapeutic effect of leaf extract of the plant has been reportedly linked with its constituent (Njoku and Akumefula , 2007). Saponin, one of the constituents, has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in female animals and the subsequent release of milk (Okwu and Okwu, 2004).

Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity (Gwotmut and Nwafor, 2001). These phytochemicals may be associated with the reported oxytocic and abortifacient activity of the plant's leaf extract (Offiah and Anyanwu, 1989). This is why the leaves of Spondias Mombin are given to expectant ruminant animals and those that delivered without the release of their placenta (Okwu and Ekeke, 2003). Other constituents include Flavonoids and some other Phenolic derivatives, Alkaloids, and Tannins. Tannins has astringent properties. It hastens the healing of wounds and inflamed mucous membrane. Flavonoids, alkaloids and tannins observed in the plant have also been associated with the observed antimicrobial effects in various studies involving plant extracts (Nwaogu *et al,* 2007). These also account for the plant's reported molluscicidal (Corthout *et al,* 1994), anti-viral (Corthout *et al,* 1992), anti-malarial (Caraballo *et al,* 2004) and anti-helminthic (Ademola *et al,* 2005) activities. Major nutritional compositions of *S. mombin* leaves include carbohydrates, moisture, proteins and crude fibre and vitamins C and A. The good distribution of nutrients in the leaves may explain its use as one of the forage feed given to domestic animals. When compared with some other common vegetables domestic animals graze on, *S. Mombin* leaves contain fairly good quantities of carbohydrates (68.92%), proteins (11.04%) and fats (4.82%). Intake of the plant for these various uses do not take into cognizance the effect it might have on the reproductive pattern of the animas.

The reproductive cycle of female wistar rats is characterized as proestrus, oestrus, metoestrus and dioestrus. Noakes *et al* 2001). In studies about reproductive system as well as studies about the influence of the estrous cycle on non-reproductive functions , vaginal smear cytology is used for the determination of the estrous cycle phases (Long & Evans, 1922). The haracterization of each phase is based on the proportion among three types of

cells observed in the vaginal smear: epithelial cells, cornified cells and leukocytes. From the onset of sexual maturity up to the age of 12 months, the mean cycle length in the female rat is 4 days (Noakes *et al* 2001), and this short cycle length makes the rat an ideal animal for investigation of changes occurring during the reproductive cycle (Caligioni 2009).

This work was carried out to evaluate the effect ethanolic extract of spondias mombin leaves has on the vaginal cytology pattern of rats treated with graded doses and ultimately on litter size and birth weight of pups.

Materials and Method

Plant Collection

Fresh leaves of Spondias mombin plant were air-dried under room temperature until they crumbled to touch. The dried leaves were grounded into fine powder, defattened with hexane before soaking in ethanol for three days. The resultant filtrate was distilled in rota-evaporator.

Experimental Animals

Twenty-five virgin female wistar rats weighing between 120g and 200g and five proven male were used for the study. The rats were fed with commercially prepared feed and water ad-libitum.

Experimental Design

Rats were allowed to acclimatize for two weeks. Female rats were divided into five groups (A, B, C, D, and E) of five rats per group. Group E served as control while the other groups (A, B, C, and D) served as experimental groups. They were treated with graded oral dosages of 800mg/kg, 600mg/kg, 400mg/kg and 200mg/kg extract and distilled water for groups A, B, C, D and E respectively.

Vaginal Cytology Preparation

This was done on the female rats daily for two weeks before treatment with extract to establish an oestrous cycle pattern. This was repeated daily for another 2 weeks simultaneously with administration of the extract. This procedure was done by inserting vaginal swab into the vaginal to make vaginal smear on a clean glass slide, fixed with methanol for 10 minutes and then stained with Giemsa for 15 minutes. This was then washed, air dried and viewed under light microscope at x100 magnification for vaginal epithelial cell types.

Mating and Pregnancy Diagnosis

A proven male was introduced to each group after 3 weeks of extract administration. Successful mating was ascertained by the presence of a copulatory (vaginal) plug on the floor of the cage the next morning and/or the presence of sperm cells in fresh vaginal smear made on clean microscope slide and observed under the x10 magnification of wide angle eyepiece of the light microscope.

Pregnancy diagnosis was done through observation of vaginal plug and abdominal palpation.

Determination of Birth Parameter

Gestation length, Litter size, Live birth weights using a sensitive electronic weighing device were taken

Statistical Analysis

Statistical analyses were carried out using Analysis of variance (ANOVA) and the means separated using Duncan's New Multiple Range Test. Data are presented as the mean ± standard deviation.

Result

The exfoliative vaginal cytology of female rat revealed that all the female rats cycled normally from one phase of oestrous cycle to another. This consistent cycle pattern was observed for both pre treatment cytology and cytology during treatment. (Fig 1) (Plates 1-4)

Pregnancy and Birth rate were 80% for groups A and E (control), 60% for groups B and C and dismal 40% for group D. (Fig 2)

Average litter sizes in all the groups were not significantly different (Table 1). The average live birth weight of the neonates measured was observed to be highest for Group A with average live birth weight of 6.27g, followed by Groups B and the control with average live birth weight of 5.83g and 5.50g. The Groups C and D had lower average live birth weights of 4.01g and 4.74g respectively.(Table

Table 1: Shows average litter size, gestational length and pups' weight for each group

GROUP S	AVERAGE LITTER SIZE (+SD)	AVERAGE GESTATIONAL LENGTH (+SD)	AVERAGE PUPS' WEIGHT (g) (+SD)
A (800 mg/kg)	6.67 ±1.15	24.33±2.52	6.27 ± 0.45
B(600mg/kg)	7.67 ± 0.58	23.00 ± 1.00	5.83 ± 0.55
C(400mg/kg)	6.75± 0.50	22.67±0.58	4.01 ± 0.26
D(200mg/kg)	6.33 ±1.15	20.5 ± 0.71	4.74 ± 0.54
E(Distilled water)	8.00 ± 2.00	21.33 ± 1.53	5.50 ± 0.67

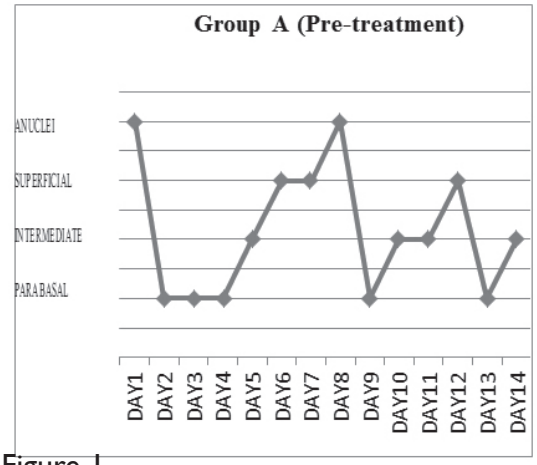
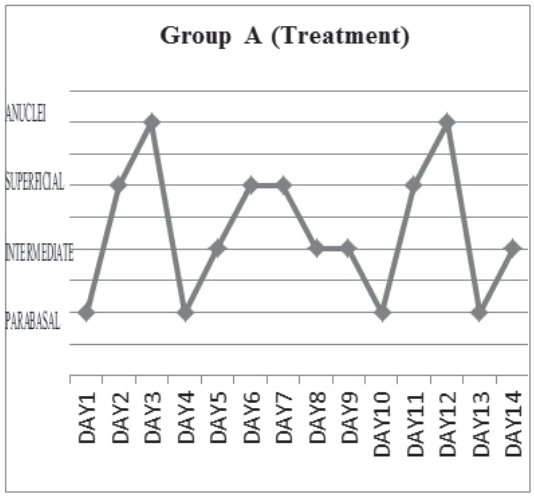


Figure 1



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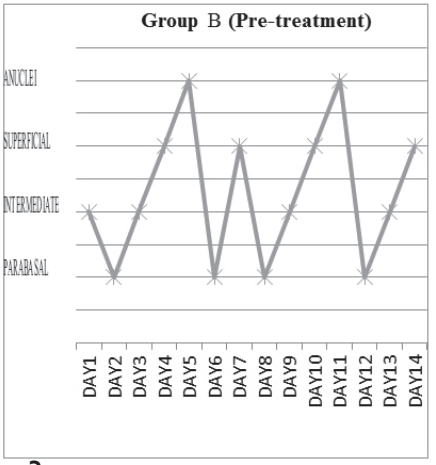

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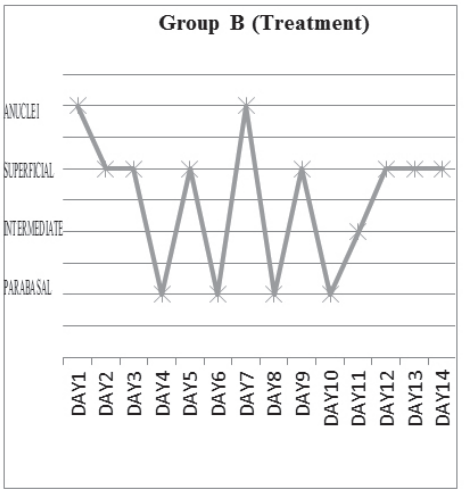

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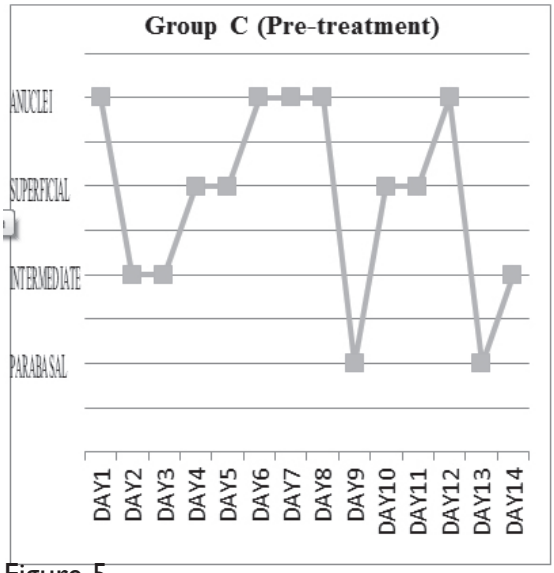

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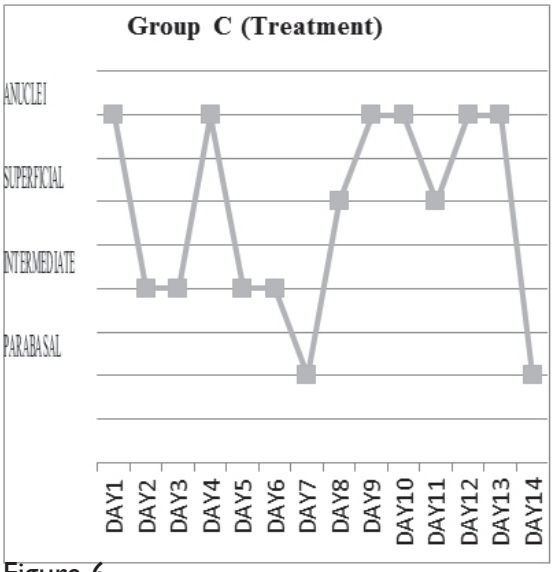

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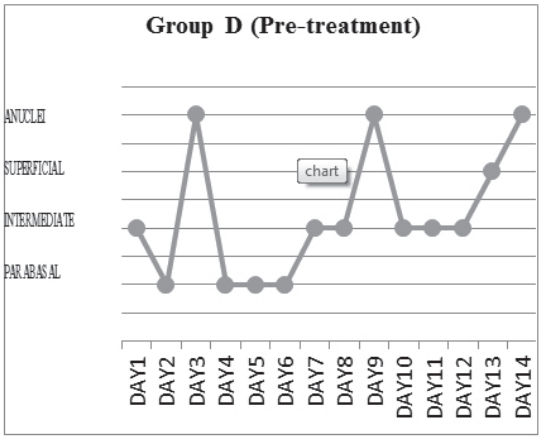

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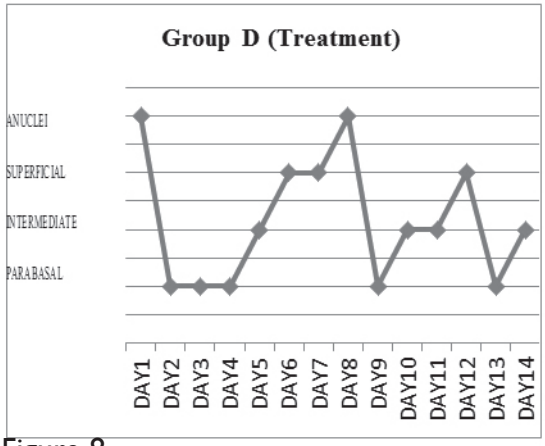

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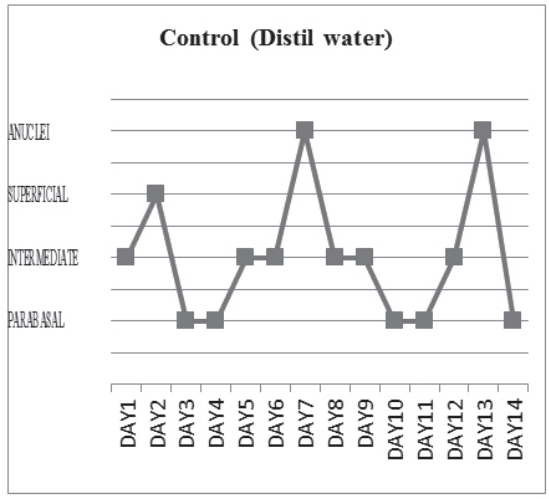

Figure 9

Figure1-9: Show predominant vaginal epithelial cell of non treated and treated period in the groups

1). The group A average weight was significantly high compared with other treatment groups and control ($p<0.05$).

Discussion and Conclusion

The experiment showed no effect of the extract on oestrous cycle pattern comparing pre treatment with treatment. It has been reported that during preclinical investigations into the safety of drugs and chemicals, many are found to interfere with reproductive function in the female rat. This interference is commonly expressed as a change in normal morphology of the reproductive tract or a disturbance

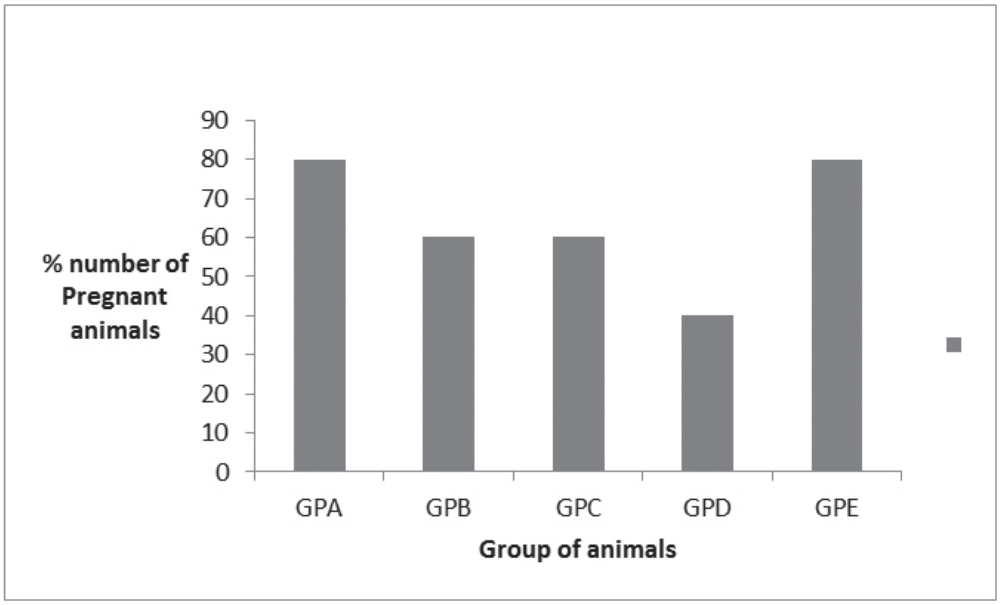

Figure 10: Shows the various percentage of pregnant after treatment with Ethanolic extract of Spondias mombin of different dosage

in the duration of particular phases of the estrous cycle. (Westwood, 2008). There seems not to be any interference with the cycle of the rats during treatment going by the wave-like consistent pattern of oestrous cycle from the proestrus phase to the diestrus phase .

The average birth weight of 5.49g and 5.83g for Group E (Control) and Group B (600mg/kg) respectively was in line with 5-6g reported by National laboratory animal centre (2010), however Group A's higher average birth weight of 6.27g even with the sizeable average number of pups. Litter sizes of all groups fell slightly below 9-11 pups reported by National laboratory animal centre (2010) the same with average Gestation lengths which fell slightly outside the range of 19-22 days reported. The profertility indications at 800mg/kg using oral dosages of ethanol exrtract of spondias mombin is against the finding of Chukwuka and Thomas (2008) working with aqueous ethanol leaf extract of Spondias mombin administered intraperitoneally. The 800mg/kg dosage was concluded as possessing anticonceptive activity. The difference in these findings could be as a result of difference in the route of administration. The choice of oral route in this work was informed by the fact that dosing of livestock using spondias leaves' extract are more often through the oral route.

It can be concluded that Ethanolic extract of Spondia Mombin at 800mg/kg fed orally before copulation has no anti fertility effect on female wistar rat rather it appears to potentiate reproduction. The same cannot be said for dosages as low as 200mg/kg.

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EFFET DE LA SUBSTITUTION DU MAÏS PAR LE MANIOC DANS L'ALIMENT SUR LES PERFORMANCES DE CROISSANCE ET LES CARACTÉRISTIQUES DE LA CARCASSE DE LA POULE LOCALE DU CAMEROUN.

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Résumé

Un essai a été mené dans le but de tester l'effet de la farine de manioc comme source d'énergie sur les performances de croissance de la poule locale. A cet effet 192 poussins d'un jour non sexés ont été repartis dans 16 unités expérimentales suivant un dispositif complètement randomisé comportant 4 traitements et 4 répétitions. A chaque phase de croissance, quatre rations expérimentales (R0) 0%, (R1) 33%, (R2) 66% et (R3) 100% de farine de manioc en remplacent du maïs ont été testées. Les données sur la consommation alimentaire, l'évolution du poids vifs et les caractéristiques de la carcasse des mâles à l'âge de 20 semaines ont été collectées.

Les principaux résultats ont montré que l'utilisation de la farine de manioc comme source d'énergie chez la poule locale n'affecte pas négativement (P>0,05) les paramètres de croissance en phase finition quelque soit le sexe considéré. Par contre, au démarrage, une baisse significative (P<0,05) de la consommation alimentaire a été enregistrée avec 33% de manioc (2991g) en substitution du maïs comparée à celle de la ration témoin (3515g). L'indice de consommation cumulé a été significativement (P<0,05) moins élevé avec les rations R1 et R2 (3,96 et 4,01) respectivement comparées à la ration témoin (R0) sans manioc (4,58). Le coût de production à douze semaines pour les deux sexes confondus a été significativement (P<0,05) plus élevé avec la ration témoin sans manioc R0 (1031,06F CFA) comparé aux rations R1 et R2 (891,54 et 909,99F CFA). Le poids de l'intestin a été significativement (P<0,05) moins élevé avec la ration R0 (29,14g) sans manioc comparé à celui de la ration contenant exclusivement du manioc (37,83g). La densité de l'intestin a été significativement (P<0,05) moins élevée avec les rations R0 et R1 (0,25 – 0,26g/cm) contenant respectivement 0 et 33% de manioc en substitution du maïs comparé à la ration R3 (0,30g/cm) avec 100% de manioc. Les caractéristiques de la carcasse n'ont pas été affectées de manière significative (P>0,05) par l'incorporation du manioc dans l'aliment. Il a été conclu dans les conditions de la présente étude, que jusqu'à 100% de maïs peuvent être remplacé par de la farine de manioc dans l'aliment de la poule locale sans affecter négativement leurs performances de croissance comparé à celle des animaux nourris exclusivement de maïs.

Mots clés: Energie alimentaire, Farine de manioc, Maïs, Performances de croissance, Poule locale.

EFFECT OF REPLACING MAIZE WITH CASSAVA ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF LOCAL HENS IN CAMEROON

Abstract

A study was conducted to evaluate the effect of cassava flour as a source of energy on local hen growth performance. For this purpose, 192 day-old unsexed birds were allotted to 16 experimental units in a completely randomized design comprising 4 treatments and 4 replicates each. Four experimental rations in which 0% (R0), 33% (R1), 66% (R2), 100% (R3) of maize was replaced with cassava flour were tested. The data on feed consumption, live weight, and male carcass characteristics at 20 weeks of age were collected. The main results indicated that the incorporation of cassava flour in the diet as energy source did not induce any significant negative effect (P>0.05) on growth parameters. However, during the brooding period, consumption of the R1 diet (2991g) was significantly (P<0.05) lower compared to the control diet (3515g). The feed conversion ratio was significantly (P<0.05) lower (3.96- 4.01) with the diets R1 and R2 respectively compared to the control (4.58). The production cost at twelve weeks was lower (891.54 –

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909.99 FCFA) with the diets with 33% and 66% substitution of maize with cassava flour as compared to the control diet (1031.06 FCFA). The intestine was significantly (P<0.05) lighter (29.14g) with the control diet compared to the one with no maize (37.83). The density of the intestine with the control and the R1 diets was significantly (P<0.05) lower, (0.25 -0.26g/cm) than that of the diet with no maize (0.30g/cm). The carcass characteristics where not been significantly (P>0.05) affected by the substitution of maize with cassava flour in the diet. It was concluded in the conditions of the present study that, up to 100% maize could be replaced with cassava flour as energy source in the diet of local chicken without any negative effect on growth performance.

Key words: Cassava root meal, Maize, Feed energy, Growth performance, Local chicken.

Introduction

La volaille africaine est un réservoir de gènes dont la valeur est à la hauteur de son adaptation aux conditions tropicales. Elle est constituée d'un génotype lui permettant de résister au climat difficile et de s'adapter à divers types d'aliments. Composée essentiellement de poules (Gallus domesticus), cette volaille est élevée dans un système extensif en milieu rural et péri-urbain et répond mieux aux méthodes culinaires et aux goûts des populations africaines (Kperegbeyi *et al.,* 2009). Les déficits en protéines animales enregistrés en Afrique subsaharienne sont surtout liés aux faibles niveaux de productivité de cette volaille qui représente plus de 50% du cheptel soit approximativement 94% de l'élevage avicole total au Nigeria (Tadelle *et al.,* 2000; Alabi et Aruna, 2006), 70% en Côte d'Ivoire et 56% au Cameroun (FAO, 2008).

Le coût élevé des ingrédients qui entrent dans la composition des aliments de commerce ainsi que les contraintes d'approvisionnement limitent leur utilisation au niveau villageois. Le prix du maïs est l'une des principales contraintes qu'il faudrait lever car, ingrédient de base, il entre pour près de 70% dans la composition des aliments de la volaille (ITAVI, 2002).

Au regard de l'importance socio-économique de la poule locale et de son rôle dans les habitudes alimentaires des populations africaines, il est nécessaire d'envisager l'intensification de sa production à partir des ressources alimentaires bon marché et surtout disponibles localement et en toutes saisons.

En raison de sa forte production, de son adaptation aux différentes conditions climatiques et pédologiques, le manioc (Manihot esculenta) est produit dans plusieurs zones agro-écologiques de l'Afrique. Son incorporation dans l'aliment du poulet de chair à un taux de 50% en substitution du maïs n'a eu en général aucun effet négatif sur la productivité (Tiemoko, 1995; Khajarern et Khajarern, 2007; Adeyemi *et al.,* 2008; Dahouda *et al.,* 2009; Manfouo *et al.,* 2010).

L'objectif de la présente étude est de contribuer à l'utilisation du manioc (Manihot esculenta) comme source d'énergie alternative au maïs (Zea mays) dans l'alimentation de la poule locale (Gallus domesticus) du Cameroun.

Matériel et Méthode

Un total de 27 poules et 3 coqs de plumage identique (coucou) achetés sur le marché de la ville de Dschang ont été mis en reproduction (neuf poules pour un mâle) à la Ferme d'Application et de Recherche de la Faculté d'Agronomie et de Sciences Agricole de l'Université de Dschang au Cameroun entre Novembre 2010 et Août 2011. Des œufs ont été collectés et mise en incubation artificielle. 192 poussins d'un jour pesant en moyenne 33g ont été obtenus et repartis en 16 unités expérimentales de 12 sujets chacun.

Les animaux ont été élevés en phase démarrage à une densité de 8 animaux/m2 pendant 12 semaines. Au terme de cette période, ils ont été séparés en fonction du sexe puis élevés en phase croissance (13 à 20 semaines) à une densité de 8 sujets/m2.

Les poussins ont été vaccinés contre la maladie de Newcastle et la Bronchite infectieuse le 7ème jour avec un rappel le 23ème jour et contre la maladie de Gomboro le 10ème jour. Des vitamines et un anticoccidien ont été administrés dans l'eau de boisson trois jours de suite toutes les deux semaines pendant la phase démarrage. Un anticoccidien

et un déparasitant interne ont été administrés toutes les trois semaines pendant les phases croissance. Les animaux ont été suivis et soignés chaque fois que la nécessité s'imposait.

La farine de manioc utilisée dans cette étude était des cossettes destinées à l'alimentation humaine et vendues sur les marchés locaux. La composition des aliments a été faite à partir des besoins théoriques des poules répertoriés dans les tables de la composition des aliments. A chaque phase (démarrage et croissance), quatre rations expérimentales comportant respectivement 0% (R0), 33% (R1), 66% (R2) et 100% (R3) de manioc en remplacement du maïs ont été fabriqués (Tableaux 1). Chacune de ces rations expérimentales a été attribuée au hasard à quatre unités expérimentales suivant un dispositif complètement randomisé comportant quatre traitements et quatre répétitions. L'aliment et l'eau ont été servis ad libitum.

Collecte des données

Les animaux ont été pesés le premier jour et tous les sept jours par la suite, en même temps que les aliments. Pendant les deux premières semaines, les poussins ont été pesés en groupe par unité expérimentale. Par la suite, des bagues d'identification ont permis des pesées individuelles. Le gain de poids hebdomadaire a été obtenu en faisant la différence entre deux poids hebdomadaires consécutifs.

A l'âge de vingt semaines, trois coqs par unité expérimentale, soient 12 sujets par traitement ont été choisi au hasard, mis en diète alimentaire pendant 24 heures puis pesés, saignés, plumés et éviscérés tel que préconisé par Jourdain (1980). Le poids de la carcasse et des différents organes à été pris à l'aide d'une balance électronique de haute précision. La longueur de l'intestin a été mesurée de la loupe duodénale jusqu'au caecum à l'aide d'un mètre ruban.

Le coût de production du kg du vif de la poule a été calculé, en multipliant le coût du kg de l'aliment par l'indice de consommation. Le prix du kg d'aliment a été évalué sur la base du prix des ingrédients sur le marché au moment de l'étude.

Analyse statistique

Les données relatives aux paramètres de croissance, aux caractéristiques de la carcasse et au coût de production du kg de poids vif ont été soumises à l'analyse de la variance à un facteur. Une séparation des moyennes a été faite avec le test de Ducan quand leurs différences étaient significatives à l'aide du logiciel S.P.S.S. 12.0.

Résultats

L'incorporation de niveaux croissants de manioc dans l'aliment tend à baisser la consommation alimentaire au démarrage (1 à 12 semaines). Toutefois, cette baisse n'a été significative ($P<0,05$) qu'entre la ration témoin (R0) sans manioc (3515g) et la ration R1 contenant 33% de substitution de maïs par du manioc (2991g) (Tableau 2). Par contre, en croissance (13 à 20 semaines), l'analyse statistique n'a révélé aucune différence significative ($P>0,05$) entre les traitements pour ce paramètre quel que soit le sexe considéré et indépendamment du sexe.

Quels que soient l'âge et le sexe considérés, l'incorporation du manioc dans l'aliment n'a eu aucun effet significatif ($P>0,05$) entre les traitements en ce qui concerne le poids vif et le gain de poids. Les courbes de l'évolution hebdomadaire du poids vif chez le mâle (Figure 1) et chez les femelles (Figures 2) ont la même allure. Cependant, alors que toutes les courbes tendent à se confondre chez les mâles, chez les femelles la courbe de poids du traitement R3 avec 100% manioc est en dessous de celles des autres traitements sur toute la période de l'étude. Par ailleurs, les courbes de poids des femelles atteignent leur point d'inflexion à la 19ème semaine pendant que celles des mâles sont ascendantes sur toute la période de l'étude. Quels que soient le sexe et la phase de l'étude considérés, aucune différence significative ($P>0,05$) n'a été enregistrée entre les groupes d'animaux pour le poids vif.

Au démarrage (1 à 12 semaines) l'indice de consommation cumulé a été significativement ($P<0,05$) moins élevé (3,96 - 4,01) avec les rations contenant 33 % (R1) et 66% (R2) de substitution comparés à la

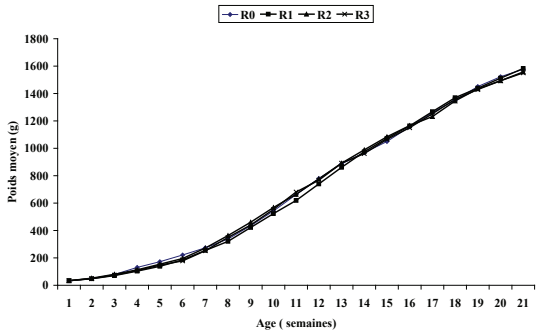

Figure 1: Effet du taux d'incorporation de la farine de manioc dans l'aliment sur l'évolution hebdomadaire du poids vif des coquelets de race locale du Cameroun

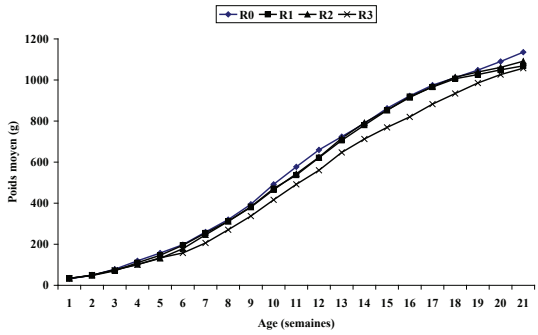

Figure 2: Effet du taux d'incorporation de la farine de manioc dans l'aliment sur l'évolution hebdomadaire du poids vif moyen des poulettes de race locale du Cameroun

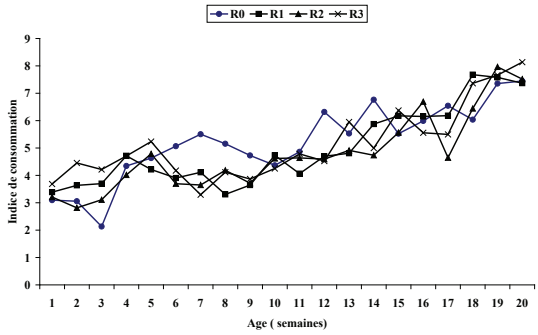

Figure 3: Effet du taux d'incorporation de la farine de manioc dans l'aliment sur l'évolution hebdomadaire de l'indice consommation des coquelets locaux du Cameroun

ration témoin (R0) sans manioc (4,58). Toutes les rations contenant de manioc (R1, R2 et R3) ont été comparables (P>0,05) pour ce paramètre d'une part et d'autre part, il n'y a pas eu de différence significative (P>0,05) entre la ration témoin R0 et la ration R3 contenant 100% de substitution du maïs par le manioc (Tableau 2). En période de croissance (13 à 20 semaines) tous les groupes d'animaux ont été comparables (P>0,05) pour l'indice de consommation quelque soit le sexe considéré et indépendamment du sexe.

Lévolution de l'indice de consommation hebdomadaire est ascendante aussi bien chez les coquelets (Figure 3) que chez les poulettes (Figure 4) et quel que soit le taux d'incorporation de la farine de manioc dans l'aliment. Ce paramètre a varié entre 3 et 8 entre la 1ère et la 20ème semaine. On note cependant que ce paramètre a évolué en dents de scie dans tous les groupes d'animaux.

Le coût de production du kg de poids vifs à douze semaines d'âge pour les deux sexes (Tableau 2) a été significativement plus élevé (P<0,05) chez les animaux de la ration R0 sans manioc (1031,06 F CFA) comparé aux rations R1 (891,54 F CFA) et R2 (909,99 F CFA) avec respectivement 33% et 66% de substitution du maïs par le manioc. Pour la ration contenant 100% de substitution, le coût de production (945,72 F CFA) a été comparable (P>0,05) à celui de la ration témoin R0 (1031,06 F CFA). De même le coût de production tend à augmenter avec le taux d'incorporation du manioc même si les trois rations R1, R2 et R3 contenant du manioc ont été comparables (P>0,05) pour ce paramètre.

Chez les mâles, à l'âge de vingt semaines, le coût de production le plus élevé a été enregistré avec la ration R3 (1348,29 F CFA) contenant 100% de manioc et le plus faible a été enregistré avec la ration R2 (1192, 41 F CFA) contenant 66% de manioc en substitution du maïs. Chez les femelles c'est plutôt avec la ration R2 (1567,90 F CFA) dans laquelle 66% de maïs ont été remplacés par du manioc que le coût le plus élevé a été enregistré alors que le plus faible a été enregistré avec la ration R1(1436,46 F CFA) contenant 33% de manioc. Cependant, aucune différence significative (P>0,05) n'a été révélée entre les traitements pour le coût de production quelque soit le sexe et indépendamment du sexe.

De manière générale, on note du Tableau 3 qui résume l'effet des traitements sur la croissance des organes de digestion

Tableau 1: Composition, valeur nutritive et coût de production des rations expérimentales

Ingrédients (kg)	**Démarrage**				**Croissance**			
	R0 (0%)	**R1 (33%)**	**R2 (66%)**	**R3 (100%)**	**R0 (0%)**	**R1 (33%)**	**R2 (66%)**	**R3 (100%)**
Maïs	48	32	16	0	48	32	16	0
Manioc	0	16	32	48	0	16	32	48
Remoulage de Blé	15,5	13	10,5	8	22,5	18	15	10
Tourteaux de coton	8	8	8	8	8	8	8	8
Tourteau de soja 49	15.5	15,5	16,5	17,5	8,5	12	15	18,5
Farine de sang	1	2,5	3,5	4,5	0	0	0	0
Farine de poisson	5	5	5	5	5	5	5	5
Poudre de coquillage	1,5	1,5	1	0,5	2	1,5	0,5	0,5
Concentré 5% (*)	5	5	5	5	5	5	5	5
Huile de palme rouge	0,5	1,5	2,5	3,5	1	2,5	3,5	5
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Valeurs nutritives calculées								
Protéine brute (%) Energie	23,17	23,08	23,06	23,04	20,09	20,1	20,12	20,06
métabolisable (kcal/kg)	2968,1	2962,9	2964,9	2966,7	3012,9	3014,5	3014,7	3000,5
Calcium (%)	1,37	1,40	1,24	1,08	1,55	1,39	1,04	1,07
Phosphore (%)	0,58	0,56	0,55	0,53	0,61	0,59	0,57	0,54
Lysine (%)	1,38	1,45	1,51	1,57	1,13	1,18	1,22	1,27
Méthionine (%)	0,48	0,47	0,46	0,45	0,44	0,43	0,43	0,42
EM/PB	128,1	128,4	128,6	128,8	149,9	149,9	149,8	149,6
Prix (Fcfa/kg)	224	225	226	228	202	210	213	221

*Concentré 5%: Protéine brute = 40%; Energie métabolisable = 2078 kcal/kg; Calcium = 8% ; Phosphore disponible = 2,05% ; Lysine = 3,30% ; Méthionine = 2,40%

Tableau 2: Effet du taux d'incorporation de la farine de manioc dans l'aliment sur les performances de croissance de la poule de race locale

Période (semaines)		**Rations**				**P**
		R0 (0%)	**R1 (33%)**	**R2 (66%)**	**R3 (100%)**	
Consommation alimentaire (g)						
1 à 12	Male, Female	3515±16^{b}	2991±264^{a}	3083±272^{ab}	3082±357 $^{a\ b}$	
13 à 20	Male	4230 ± 556	4419±264	3701±413	4009±769	NS
	Female	3000±519	2444±242	2730±431	2738±341	NS
	Male, Female	3615± 343	3431±224	3216±268	3373±514	NS
Indice de consommation						
1 à 12	Male, Female	4,58 ± 0,43 ^{b}	3,96 ± 0,32 ^{a}	3,96 ± 0,32 ^{a}	4,14 ± 0,26^{ab}	
	Male	6,16 ± 0,30	6,13 ± 0,19	5,58 ± 0,78	6,09 ± 0,28	NS
	Female	7,31 ± 0,44	6,84 ± 0,95	7,34 ± 0,45	6,69 ± 0,25	NS
	Male, Female	6,94 ± 0,34	6,90 ± 0,41	6,82 ± 0,36	6,72 ± 0,29	NS
Coût de production du kg de poids vif (FCFA)						
1 à 12	Male, Female	1031±97 b	891,54±73,8^{a}	909,99 ± 38,73^{a}	945,72± 60,69^{ab}	

13 à 20	Male	1246±60,7	1288±41,9	1192±166,8	1348± 62,5	NS
	Female	1479± 90,3	1436±200	1568±97,2	1480±56,5	NS
	Male, Female	1362±63,8	1362±113	1380±114,5	1414±44,7	NS

[a,b] Les moyennes portant la même lettre sur la même ligne ne sont pas significativement différentes (P>0,05). NS: non significatif

Tableau 3: Effet du taux d'incorporation de la farine de manioc dans l'aliment sur la croissance des organes de digestion des coqs

Organes	**Rations**			
	R0 (0%)	**R1 (33%)**	**R2 (66%)**	**R3 (100%)**
Poids relatif du gésier (%)	1,40 ± 0,18 [a]	1,39 ± 0,32 [a]	1,27 ± 0,08 [a]	1,51 ± 0,17 [a]
Poids de l'intestin (g)	29,14 ± 4,58 [a]	31,91 ± 0,99 [ab]	33,37 ± 1,63 [ab]	37,83 ± 6,54 [b]
Longueur de l'intestin (cm)	115,83 ± 13,30 [a]	122,16 ± 6,73 [a]	124,79 ± 5,12 [a]	123,83 ± 3,69 [a]
Densité de l'intestin (g /cm)	0,25 ± 0,02 [a]	0,26 ± 0,01 [a]	0,27 ± 0,01 [ab]	0,30 ± 0,04 [b]

[a,b] Les moyennes portant la même lettre sur la même ligne ne sont pas significativement différentes (P>0,05)

Tableau 4: Effet du taux d'incorporation de la farine de manioc dans l'aliment sur le rendement carcasse (%) et le poids relatif des organes (%) des coqs

Paramètres (% du poids vif)	**Rations**				
	R0 (0%)	**R1 (33%)**	**R2 (66%)**	**R3 (100%)**	**P**
Rendement carcasse (%)	71,45 ± 1,89	71,49 ± 1,05	73,20 ± 0,97	71,14 ± 1,63	NS
Foie (%)	1,15 ± 0,10	1,19 ± 0,12	1,14 ± 0,08	1,25 ± 0,91	NS
Cœur (%)	0,42 ± 0,02	0,42 ± 0,04	0,41 ± 0,04	0,39 ± 0,02	NS
Pancréas (%)	0,13 ± 0,01	0,13 ± 0,01	0,14 ± 0,02	0,14 ± 0,03	NS
graisse (%)	0,12 ± 0,17	0,14 ± 0,08	0,21 ± 0,20	0,10 ± 0,12	NS
Tête (%)	3,42 ± 0,22	3,42 ± 0,14	3,60 ± 0,33	3,56 ± 0,37	NS
Pattes (%)	3,54 ± 0,19	3,63 ± 0,14	3,46 ± 0,10	3,67 ± 0,22	NS

NS : non significatif

que les différents traitements n'ont eu aucun effet significatif (P>0,05) sur le poids relatif du gésier. Il en est de même pour la longueur de l'intestin même si les rations contenant du manioc ont dans l'ensemble induit des intestins plus longs. Par contre avec 100% de substitution du maïs par le manioc (R3) dans l'aliment comme source d'énergie, le poids de l'intestin a été significativement (P<0,05) plus élevés (37,83g) par rapport à la ration témoin sans manioc (29,14g). Les trois rations R1, R2 et R3 contenant du manioc ont été comparables (P>0,05) pour ce paramètre avec des poids respectifs de 31,91 ; 33,37 et 37,83g. La densité de l'intestin a été significativement (P<0,05) moins élevée avec les rations R0 (0,25g/cm) et R1 (0,26g/cm) respectivement avec 0% et 33% de manioc comparées à la ration R3 (0,30g/cm) contenant 100% de manioc. Les rations R2 et R3 contenant respectivement 66 et 100% manioc en remplacement du maïs ont été comparables (P>0,05) pour ce paramètre.

L'effet de l'incorporation du manioc dans l'aliment sur le rendement carcasse, le poids relatif du foie, du cœur, du pancréas, de la graisse abdominale, de la tête et des pattes des coqs à l'âge de vingt semaines est résumé dans le Tableau 4. L'analyse statistique n'a révélé aucune différence significative (P>0,05) entre les traitements pour tous

les paramètres étudiés même si on peut remarquer que l'incorporation de 100% de manioc en substitution du maïs dans la ration tend à augmenter le poids relatif du foie (1,25 %) comparé aux autres groupes de coqs (1,15; 1,19 et 1,14 respectivement pour les rations R0, R1 et R2). De même les traitements R2 et R3 (0,14) contenant 66 et 100% de manioc ont été comparables aux rations R0 et R1 (0,13) contenant respectivement 0 et 33% de manioc pour le poids relatif du pancréas.

Discussion

L'utilisation de la farine du manioc comme source d'énergie dans l'alimentation de la poule locale a induit une baisse significative ($P<0,05$) de la consommation alimentaire cumulée au démarrage (0 à 12 semaines) comparé à la ration sans manioc. Ce résultat corrobore ceux de Khajarern et Khajarern (2007) qui ont rapporté que la palatabilité des rations à base de manioc et la texture poudreuse de cet ingrédient sont des facteurs pouvant limiter la consommation alimentaire chez la poule. De même, Ojelewa et al. (2006) ont rapporté que la taille des particules de manioc affecte négativement la consommation alimentaire. C'est dire que la finesse de la mouture du manioc serait un véritable frein pour son utilisation en aviculture. Par contre Ukachukwu (2005) avait enregistré une consommation plus élevée avec des rations à base de farine de manioc par rapport au témoin sans manioc. La différence entre ces résultats serait due non seulement aux souches utilisées, mais aussi aux ingrédients auxquels est associée la farine manioc dans ces différentes études.

L'incorporation de la farine de manioc dans l'aliment n'a pas eu d'effet significatif ($P>0,05$) sur le poids vif et sur le gain de poids. Cependant, on a observé, en ce qui concerne le poids vif et plus particulièrement au niveau des femelles, une tendance à la baisse avec des taux croissants de manioc dans l'aliment. Cette tendance à une croissance plus lente concorde avec les observations de Dahouda *et al.* (2009) qui ont conclu que la substitution partielle du maïs avec des sous-produits du manioc affecte négativement la croissance des pintades. Dans le même ordre d'idée, Salami et Odunsi (2003) ont substitué 50 %, 75 % et 100 % de maïs avec des cossettes de manioc dans l'aliment des poules pondeuses et ont observé qu'avec une substitution de 75 % de maïs, le poids final de 1,55 kg obtenu était significativement inférieur à celui du lot témoin (1,93 kg) et que, au-delà de 75 %, la chute du poids était beaucoup plus importante.

L'indice de consommation a diminué avec le taux d'incorporation du manioc dans l'aliment au démarrage. Ce résultat est similaire à celui d'Akinfala *et al.* (2002) sur des poulets de chair au Nigeria qui ont enregistré des indices de consommation plus faibles avec des rations contenant du manioc comparés à la ration témoin. Par contre, ce résultat est contraire à celui de Chhum (2004), qui a rapporté des indices de consommation plus élevé avec les rations contenant du manioc dans une étude portant sur l'amélioration de la productivité du poulet de chair au Cambodge. Pendant la période de croissance, les indices de consommation ont été comparables dans tous les lots d'animaux. Ce résultat concorde avec à celui de Dahouda *et al.* (2009) qui ont rapporté des indices similaires avec différents taux d'incorporation de manioc dans l'aliment de pintades en croissance.

A l'âge de douze semaines, le coût de production du kg de poids vif à été significativement ($P<0,05$) plus élevé avec la ration sans farine de manioc (R0) par rapport aux rations R1 et R2 contenant respectivement 33% et 66% de manioc en substitution au maïs. Cette observation pourrait s'expliquer par l'effet combiné du mauvais indice de consommation de la ration sans manioc et du prix relativement bas des cossettes de manioc. Ce résultat est comparable à celui de Dahouda *et al.* (2009) qui a révélé que la production de la pintade est plus rentable avec les aliments contenant des feuilles et des cosettes de manioc qui peuvent constituer des sources alternatives d'énergie et de protéines.Au terme de vingt semaines, les coûts de production ne présentent pas de différence significatives ($P>0,05$) quelque soit le traitement et le sexe considéré et indépendamment du sexe. Ce résultat confirme les conclusions d'Akinfala *et al.* (2002) selon les quelles l'utilisation

du manioc en remplacement du maïs dans l'alimentation ne peut être économique qu'en période où cet aliment est disponible à un prix compétitif.

L'introduction du manioc dans l'aliment de la poule locale tend à augmenter significativement ($P<0,05$) le poids et la densité de l'intestin. Ce qui suppose une augmentation de la surface d'absorption des nutriments et une utilisation plus adéquate de ceux-ci. Le manioc ayant un taux de cellulose relativement plus élevé que celui du maïs, cette observation confirmerait les explications données par Thorburn et Wilcox (1985) cités par Aderemi et Nworgu (2007) selon lesquelles, chez les monogastriques, un taux élevé de cellulose stimulerait la croissance et l'épaississement des parois du tractus digestif. Dans le même sens, Teguia *et al.* (2004) ont émis l'hypothèse selon laquelle l'ingestion d'une teneur élevée en cellulose augmenterait le poids du tractus digestif.

Aucune différence significative n'a été observée entre les poids des différents organes en fonction des teneurs en farine de manioc dans l'aliment. Des résultats similaires ont été obtenus par Dahouda *et al.* (2009) qui n'ont observé aucune différence entre les poids de la carcasse et des abats de pintades nourris en phase de croissance avec différents niveaux de manioc en substitution du maïs dans l'aliment. A Madagascar, les rendements carcasses de la poule locale à 5 mois situés entre 64 et 66% rapporté par Koko *et al.* (2006) sont plus faibles que ceux de la présente étude. Par ailleurs, celui enregistré au Congo par Akouango *et al.* (2010) de 78% pour le coq à 6 mois d'âge est plus élevé. Ces différences seraient dues à la variabilité génétique, aux systèmes d'élevage et surtout à l'aliment utilisé dans ces différentes études.

Conclusion

La substitution jusqu'à 100% du maïs par le manioc dans l'aliment n'a pas d'effet dépressif significatif sur les performances de croissance des poules et sur les caractéristiques de la carcasse des coqs locaux. Cette farine pourrait donc être utilisée comme source alternative d'énergie alimentaire au maïs dans l'aliment de la poule locale pendant la phase démarrage et croissance.

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EFFETS DU CHARBON DE NOYAUX DE CANARIUM SCHWEINFURTHII ENGL. OU DE RAFLES DE MAÏS SUR LES PERFORMANCES DE PRODUCTION D'ŒUFS PAR DES POULES EN FIN DE CARRIERE DE PONTE

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Resume

La présente étude avait pour objectif d'évaluer l'effet de l'incorporation des charbons de noyaux de Canarium schweinfurthii et de rafles de maïs dans l'aliment sur les performances de production d'œufs de consommation par des poules en fin de carrière de ponte. A ce titre, 200 poules de souche SHAVER 579, âgées de 62 semaines ont été reparties dans 5 groupes (traitements). Le premier groupe recevait un aliment sans charbon et constituait le groupe témoin (T). Les 4 autres groupes recevaient 0,2 ou 0,4% de charbon de noyaux de Canarium (C0,2 et C0,4) ou de rafles de maïs (R0,2 et R0,4). Les principaux résultats ont révélé que l'incorporation du charbon de rafles de maïs ou de Canarium quel que soit le taux dans l'aliment a significativement ($P<0,05$) améliorée le nombre d'œufs pondus par poule, le taux de ponte et l'indice de consommation par rapport au lot témoin. Les traitements n'ont pas affecté le poids des œufs. Dans les conditions de cette étude, il a été conclu que l'incorporation du charbon de rafles de maïs ou de noyaux de Canarium dans l'aliment des pondeuses en fin de la carrière peut améliorer de manière significative la persistance de ponte ainsi que l'indice de consommation.

Mots clés: Canarium schweinfurthii, Charbon, Œuf de table, Pondeuses, Rafles de maïs

EFFECT OF DIETARY INCLUSION OF CHARCOAL FROM CANARIUM SCHWEINFURTHII ENGL. SEEDS OR MAIZE COB ON EGG YIELD OF OLD LAYING HEN.

Abstract

This study was carried out to evaluate the effect of dietary charcoal from Canarium schweinfurthii seeds or maize cob supplementation on egg yield performances in 62 weeks old laying hens. A total of 200 SHAVER 579 laying hens were weighed and randomly allotted to five dietary treatments in a completely randomized design, each group containing 40 hens. Five experimental diets including a control and other containing either 0.2 or 0.4% charcoal from Canarium seeds (C0.2 and C0.4) or from maize cob (R0.2 and R0.4) supplements were used. Results indicate that both charcoal from Canarium and maize cob significantly ($P<0.05$) improved feed conversion ratio, egg production and persistence of laying as compared to the control group. Vegetable charcoal had no effect on egg weight. From the data obtained it can be concluding that charcoal from Canarium seeds or maize cob could be used to improve feed efficiency and persistence of egg production.

Key words: Canarium schweinfurthii, Charcoal, Layers, Maize cob, Table eggs.

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Introduction

Dans le contexte camerounais actuel, il n'est pas aisé d'augmenter la production de viande de bœuf, du fait de son extrême exigence en espace, de son cycle de production long et de sa vulnérabilité à la trypanosomiase dans certaines régions. Le poulet de chair quant à lui est encore au dessus du pouvoir d'achat du citoyen moyen dans la plupart des pays tropicaux d'Afrique. De ce fait, il est actuellement plus facile pour un individu de consommer un œuf par jour afin de couvrir ses besoins en protéine que de consommer de la viande. L'accroissement de la production des œufs de table apparaît donc comme l'un des moyens rapides susceptibles d'aider à résorber la malnutrition (Anyawale *et al.,* 2006; Ayasan *et al.,* 2006; Nwogu et Fasogbon 2008; Kakengi *et al.,* 2007; Soltan, 2008; Ugwu et Onyimonyi, 2008).

Toutefois, même si la production d'œufs s'est accrue de 12600 à 13400 tonnes/an entre 2000 et 2006 (FAO, 2008), elle reste encore très insuffisante pour couvrir les besoins des populations. Cette faible production est liée entre autre à la mauvaise qualité de l'aliment elle même liée aux mauvaises conditions climatiques qui favorisent les contaminations des matières premières ou des aliments par les bactéries et les champignons (Teguia *et al.,* 1992; Antonio *et al.,* 1996; Han *et al.,* 1999). Pour contrôler les effets néfastes des toxines produites par ces bactéries et ces champignons certains auteurs préconisent chez les poulets de chair (Anjaneyulu *et al.,* 1993; Kana *et al.,* 2010) et les canards (Ruttanavut *et al.,* 2009), l'incorporation des charbons végétaux dans l'aliment, ce qui permettrait de capter ces toxines, d'améliorer leur indice de consommation et leur vitesse de croissance. Ainsi, l'objectif de cette étude était de vérifier l'intérêt d'incorporer des charbons de noyaux de Canarium schweinfurthii (Fruits noirs) ou de rafles de maïs sur les performances de ponte des poules.

Materiels et Methodes

Site de l'étude

L'étude a été conduite à la ferme de production d'œufs de consommation de la SOCAVB (Société Camerounaise de Volaille et de Bétail) à Bomono Ba Djédu, village situé en zone côtière entre le 6e et le 8e degré latitude Nord à une altitude moyenne de 1500m dans la Région du Littoral. Le climat qui y règne est équatorial de type maritime. Les pluies sont abondantes et régulières avec une moyenne d'environ 2300mm d'eau par an. La température varie en fonction de l'altitude avec une moyenne de 30°C. L'amplitude thermique est généralement faible et l'humidité relative oscille autour de 80%.

Préparation des charbons

Les noyaux de fruits noirs matures et les rafles de maïs collectés dans les exploitations paysannes dans la Région de l'Ouest du Cameroun ont été calcinés au feu de bois et les braises ont été éteintes avec de l'eau potable. Les charbons obtenus étaient en suite séchés au soleil puis broyés dans un moulin pour en obtenir les farines de charbons qui ont été préalablement tamisées avant d'être incorporées dans les différentes rations expérimentales.

Matériel animal, rations expérimentales et dispositif expérimental

Dans cette étude, 200 pondeuses de souche SHAVER 579 âgées de 62 semaines, pesant entre 1650 et 1700g ont été reparties au hasard dans 100 cages organisées en 20 lots de 5 cages (unité expérimentale). Elles étaient logées dans des batteries de cages de type californiennes à raison de deux poules par cage. Chaque cage mesurait 47 cm de long, 34 cm de profondeur et 34 cm de hauteur. Pour éviter toute confusion, les unités expérimentales étaient séparées les unes des autres par une cage vide.

Quatre rations expérimentales ont été préparées en incorporant 0,2% ou 0,4% de charbon de noyaux de Canarium (C0,2 et C0,4) ou de rafles de maïs (R0,2 et R0,4) à la ration de base (T) qui servait de ration témoin (Tableau 1). Les animaux étaient nourris ad libitum et les refus de chaque unité expérimentale étaient pesés chaque fin de semaine afin de déterminer la consommation hebdomadaire. Chacune des 5 rations expérimentales était

attribuée au hasard à 4 unités expérimentales (répétitions) dans un dispositif aléatoire.

Collecte des données

Le poids vif des poules et la consommation alimentaire ont été évaluées tous les 15 jours jusqu'à l'age de 74 semaines. La collecte et la pesée des œufs se faisaient quotidiennement et en fin de chaque semaine, afin de déterminer le taux de ponte hebdomadaire par poule présente (TPP).

Analyse statistique

Les données sur le poids vif des poules, le nombre d'œufs pondus, le taux de ponte, le poids moyen des œufs, la consommation alimentaire et l'indice de consommation ont été soumises à l'analyse de la variance (ANOVA) suivi d'un test de Duncan en cas de différences significatives (Vilain, 1999).

Resultats

La consommation de la ration R0,4 contenant 0,4% de charbon de rafles de maïs a été significativement (P<0,05) plus élevée que celle de C0,4 contenant 0,4% de charbon de noyaux de Canarium, les autres traitements (R0,2, C0,2 et T) étant intermédiaires. Lorsque le taux d'incorporation du charbon de noyaux de Canarium augmente, la consommation tend à baisser, alors qu'elle tend plutôt à augmenter avec du charbon de rafles de maïs.

Aucune différence significative (P>0,05) n'a été enregistrée entre les traitements en ce qui concerne le poids vif des poules. Par contre, le nombre d'œuf pondus par poule et le taux de ponte ont été significativement (P<0,05) plus élevés avec les charbons par rapport au témoin. Toutefois, la différence n'a été significative (P<0,05) qu'entre les rations contenant 0,2 et 0,4% de charbon de rafles de maïs (69,70 et 71,85 œufs pondus pour un taux de ponte de 83,18 % et 85,77% respectivement) et la ration témoin (T) sans charbon (63,27 œufs pondus pour un taux de ponte de 76,23%).

La courbe de l'évolution du taux de ponte affiche la même allure dans tous les groupes. Cependant, la courbe du lot témoin est en dessous de celle de tous les lots soumis aux charbons (Figure 1). Toutefois, il est important de relever la persistance de la ponte dans tous les lots, qui à l'âge de 74 semaines est encore au dessus de 70%, largement au dessus du taux de ponte en fin de cycle, étant donné qu'en élevage des pondeuses, la réforme a lieu à partir de 55% de ponte.

Tous les traitements ont été comparables (P>0,05) pour le poids des œufs (60-61g). L'indice de consommation quant à lui baisse avec le taux d'inclusion croissant de charbon quel que soit le type considéré. Toutefois, l'indice de consommation le plus élevé (P<0,05) de tous les groupes a été enregistré chez le lot témoin (2,30) alors que le plus faible a été enregistré avec 0,4% (R0,4) de charbon de rafles de maïs (1,99).

Discussion

Cette étude a révélé que lorsque le taux d'incorporation du charbon de noyaux de Canarium augmente dans la ration, la consommation tend à baisser, alors qu'elle tend plutôt à augmenter avec du charbon de rafles de maïs. Ce résultat corrobore ceux de Kana et al. (2009) et Kana et al. (2010) qui ont enregistré une augmentation de la consommation alimentaire chez les poulets de chair avec un taux d'incorporation de ces deux charbons allant de 0,2 à 0,4% alors qu'au delà de 0,6%, ils ont enregistré une baisse significative de ce paramètre par rapport au témoin.

Le nombre d'œuf pondu et le taux de ponte ont été plus élevés avec le charbon de rafles de maïs quel que soit le taux d'incorporation dans l'aliment (69,70 et 71,85 œufs pondus pour un taux de ponte de 83,18 % et 85,77% respectivement) comparé à la ration témoin (T) sans charbon (63,27 œufs pondus pour un taux de ponte de 76,23%). Ces niveaux sont proches de ceux de Soltan (2008) qui a enregistré un taux de ponte variant entre 80 et 85% chez les poules âgés de 54 à 70 semaines alimentés avec des taux croissants d'acides organiques.

L'évolution du taux de ponte du lot témoin a été en dessous de celle de tous les lots soumis aux charbons. Ceci pourrait s'expliquer par l'amélioration de l'état sanitaire des poules,

Tableau 1: Composition de la ration de base (T)

Ingrédients	**Quantités (kg)**
Maïs	563
Tourteau de soja	50
Farine de poisson	50
Tourteau de coton	80
Son mélange	150
Coquilles	80
Prémix*	5
Farine d'os	20
Sel	2
Total	**1000**
Composition chimique calculée	
Energie métabolisable (kcal/kg)	2548,23
Protéines brutes (%)	15,77
Calcium (%)	4,04
Phosphore disponible (%)	0,75
Lysine	0,87
Méthionine	0,34

* : 5kg de premix par tonne d'aliment apporte : 30000000 IE de vit.A ; 600000 IE de vit.D3 ; 4000 IE de vit.E ; 500 mg de vit.K3 ; 200mg de vit.B1 ; 1000 mg de vit.B2 ; 2400mg d'acide pantothenique ; 7000mg de Niacine ; 10000mg de Biotine ; 3000mg de vit.B12 ; 200mg d'acide folique ; 400mg de vit.B6 ; 8000mg de Fe ; 2000mg de Cu ; 10000mg de Zn ; 14000mg de Mn ; 200mg de Co ; 200mg d'Iode ; 20mg de Se ; 201,76g de dl- Méthionine ; 20000mg de OxE310/E320/E321.

Tableau 2: Effet du taux d'incorporation des charbons de noyaux de Canarium schweinfurthii ou de rafles de maïs dans l'aliment sur les performances de production des pondeuses

Performances techniques	**T**	**$C_{0,2}$**	**$C_{0,4}$**	**$R_{0,2}$**	**$R_{0,4}$**	**P**
Consommation alimentaire (g)	11585 ±282^{ab}	11580 ± 370^{ab}	11253 ± 193^{b}	11588 ± 291^{ab}	11906 ± 148^{a}	
Poids initial des pondeuses (g)	1687 ± 52	11580 ± 370^{ab}	1643 ± 42	1700 ± 35	1693 ± 23	NS
Poids final des pondeuses (g)	1724 ± 35	1727 ± 27	1707 ± 55	1732 ± 15	1708 ± 21	NS
Nombre d'oeufs pondus par poule	63,3 ± 7,1^{b}	66,0 ± 5,0^{ab}	67,5 ± 5,4^{ab}	69,7 ± 5,5^{a}	71,9 ± 3,4^{a}	
Taux de ponte (%)	76,2 ± 7,2^{b}	78,6 ± 5,9^{b}	81,0 ± 5,4^{ab}	83,2 ± 6,1^{a}	85,8 ±4,3^{a}	
Poids moyen de l'œuf (g)	61,52 ± 1,52	60,94 ± 1,03	61,12 ± 0,78	61,06 ± 0,43	60,97 ±0,70	NS
Indice de consommation	2,30 ± 0,28^{a}	2,10 ± 0,14^{ab}	2,01 ± 0,14^{b}	2,00 ± 0,11^{b}	1,99 ± 0,11^{b}	

$^{a, b:}$ Les moyennes portant la même lettre dans la même ligne ne sont pas significativement différentes (P<0,05)
NS : Non significatif

consécutive à l'utilisation du charbon car selon Ramos *et al.* (1996), Ruttanavut *et al.* (2009) et Kana *et al.* (2010), les charbons de bois ont la capacité d'absorber les substances toxiques qui affecteraient l'utilisation des nutriments par les animaux.

Le poids des œufs enregistré dans la présente étude (60-61g) est très inférieur aux résultats de Soltan (2008) qui a rapporté un poids des œufs variant entre 64 et 66g chez des poules âgés entre 54 et 70 semaines. Par contre, cet auteur a enregistré des indices de consommation (1,97 à 2,10) très proche de ceux enregistrés dans cette étude (1,99 à 2,30).

Conclusion

Il ressort de la présente étude que l'incorporation du charbon dans l'aliment a induit une augmentation du nombre d'œufs pondus par poule présente, du taux de ponte, de la persistance de la ponte et une baisse significative de l'IC par rapport au lot témoin. Toutefois, les meilleures performances de ponte ont été enregistrées avec 0,4% de charbon de rafles de maïs. Avant de généraliser l'utilisation des charbons dans l'alimentation des pondeuses, il serait important d'étudier de manière plus approfondie leurs modes d'action.

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COMPARISON OF PROCEDURES FOR ESTIMATING MICROBIAL CONTAMINATION OF FEED RESIDUES FROM IN SITU BAGS IN SHEEP

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Abstract

In situ protein degradations of grass hay and molassed sugarbeet pulp were performed in the rumen of sheep. Three methods, namely labelling with 35S, measuring cytosine as an internal microbial marker and dissolving contaminating microbes in neutral detergent solution were compared with the aim of choosing the method that best estimated microbial contamination of in situ feed residues. Corrected protein degradation values were higher ($P<0.05$) than the values uncorrected for microbial contamination. The cytosine method gave the highest estimates of microbial presence in in situ bag residue followed by labeling with 35S. At incubation times up to 16 h, considerable quantities of dietary cytosine might have been present in the bag residue causing an overestimation of the amount of microbes present in the residue. In contrast, the neutral detergent procedure underestimated N disappearance because it removed 97.5% of the N content of solid associated microbes. Due to weaknesses of the cytosine and neutral detergent methods, the 35S technique was considered to be the best method for correcting microbial contamination of residues in the bags during in situ determination of feed degradability in the rumen.

Keywords: Microbial contamination, in situ, protein degradation, 35S, cytosine, neutral detergent fibre

COMPARAISON DES PROCEDURES D'ESTIMATION DE LA CONTAMINATION MICROBIENNE DES RESIDUS ALIMENTAIRES DANS LES SACHETS APRES UNE DEGRADATION IN SITU CHEZ LE MOUTON

Résumé

Des dégradations in situ des protéines du foin de graminées et de la pulpe de betterave sucrière mélassée ont été réalisées dans le rumen de moutons. Trois méthodes, à savoir l'étiquetage 35S, la mesure de la cytosine comme marqueur microbien interne et la dissolution des microbes contaminants dans une solution détergente neutre ont été comparées dans le but de choisir la méthode qui donnerait une meilleure estimation de la contamination microbienne des résidus alimentaires in situ. Les valeurs des dégradations de protéines corrigées étaient plus élevées ($P <0,05$) que les valeurs non corrigées pour la contamination microbienne. La méthode utilisant la cytosine a donné les estimations les plus élevées de la présence de microbes dans les résidus de sachets in situ, suivie par l'étiquetage 35S. Aux temps d'incubation allant jusqu'à 16 h, des quantités considérables de cytosine alimentaire peuvent avoir été présentes dans le résidu des sachets, causant une surestimation de la quantité de microbes présents dans le résidu. Par contre, la procédure utilisant un détergent neutre a sous-estimé la disparition de l'azote car elle a enlevé 97,5% de la teneur en azote microbiens associés aux solides. Vu les faiblesses des méthodes utilisant la cytosine et le détergent neutre, la technique 35S a été considérée comme la meilleure méthode pour corriger la contamination microbienne des résidus dans les sachets durant la détermination de la dégradabilité in situ des aliments dans le rumen.

Mots-clés: Contamination microbienne, In situ, Dégradation des protéines, 35S, Cytosine, Fibre au détergent neutre

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Introduction

The in situ method is used widely for protein degradation estimations (Shannak et al., 2000; Mohamed and Chaudhry, 2008). However, estimated protein degradability (RDP) of low nitrogen (N) feeds in situ is likely to be erroneous partly due to microbial contamination of feed residue in in situ bags (Rodriguez and Gonzalez, 2006). To improve upon the estimates, the correction of in situ residual N for the presence of microbes is relevant, particularly for feeds with low N content (Mathers and Aitchison, 1981; Michalet-Doreau and Ould-Bah, 1992).

Markers such as nucleic acids, 15NH3 and $35SO_4$ have been tried for the correction of microbial contamination of in situ residues (Broderick and Merchen, 1992; Rodriguez and Gonzalez, 2006). The method of nucleic acids is quite simple, and with the availability of high performance liquid chromatography (HPLC) instruments, the use of individual nucleic acid bases as microbial markers is being tried. The use of 35S has the advantage of less cost of analysis compared to 15N. However, the routine use of radioactivity may have serious environmental implications. Hof et al. (1990) used neutral detergent solution to rid feed material contained in in situ bags of contaminating bacteria. The neutral detergent fibre method is known to solubilize mainly the cell contents but not the cell wall. It is therefore likely to solubilize at least the cell contents of microbes attached to in situ residues. Application of the neutral detergent fibre method offers a simple and non-invasive technique.

Three methods, namely the use of a nucleic acid pyrimidine base (cytosine), neutral detergent solution and 35S were compared. The aim was to find the best method of correction for microbial contamination of feed residues.

Materials and Methods

The in situ technique and experimental feeds

The method provided by the Interdepartmental Protein Working Party (ARC, 1984) for estimating protein loss from in situ bags was used. Hay (from perennial rye grass) and molassed sugarbeet pulp (SBP) were milled separately through 4 mm screen. Each bag contained approximately 5 g air-dried sample.

Animals and Feeding

Three rumen-cannulated sheep were used in this experiment. The sheep received a maintenance level of feeding that consisted of 0.60:0.40 (air dry basis) hay: concentrate diet. The diet was consumed at two hourly intervals to help achieve steady state of 35S specific activity and of microbial labelling.

Ruminal infusion of ^{35}S

A stock solution with a concentration of 0.74 Mbq/ml (^{35}S) in the form of $Na2^{35}SO_4$ and also containing 100 µg/ml anhydrous Na_2SO_4 was made. Approximately 340 ml (containing about 251.85 MBq) of the stock solution was diluted with water to a volume of 20.6 l. Using a peristaltic pump, sheep were infused with 5.85 MBq of ^{35}S in a volume of 480 ml of water per sheep per day through the rumen cannula.

The incubation procedure

Two bags, one containing hay and the other SBP were staggered on each stalk. Incubation times were 0, 8, 16, 24, 48 and 72 h. These incubation times were duplicated for each feed within a sheep. After removal, the bags were machine washed and rinsed with cold water in a wash cycle that lasted for 24 min. Zero hour bags were subjected to 24 min. machine washing only. The residues were then analyzed for dry matter (DM), N, 35S, cytosine and neutral detergent insoluble nitrogen (NDIN) from which protein degradation and extent of microbial contamination were calculated.

Preparation of ^{35}S labelled solid-associated microbes

Rumen digesta solids were collected on four occasions from each sheep at the same time as the removal of bags from the rumen. Four samples of solid-associated microbes (SAM) were then prepared from these rumen samples (Whitehouse *et al.,* 1994). In total,

there were 12 samples of SAM (4 from each sheep) made. All the 12 samples of rumen microbes were evaluated for their N and ^{35}S contents.

Analytical procedures

^{35}S:Non-ammonia nitrogen ratio

The method provided by Mathers and Aitchison (1981) was used. The 35S:N (disintegrations/min per mg N) was computed for both microbe and residue. The proportion of microbial N in residual N was calculated as:

35S:N (residue)/35S:N (microbial).

Cytosine method

The method used was adapted from that employed by Lassalas *et al.* (1993) for the separation of purine and pyrimidine bases, including cytosine, on a chromatography system (reverse phase Ultrasphere ODS 5 µm; column, 4.6 x 250 mm; absorbance at 254 nm). Calibration curves relating amount of cytosine (y) to the area ratio of cytosine:allopurinol (x) were plotted for the calibration standard. For each sample, the concentration of cytosine was calculated using the standard calibration parameters.

The neutral detergent fibre method

The method provided by Licitra *et al.* (1996) was used.

Calculations and statistical analysis

Measurement of degradability

Fractional N disappearance of the feeds at each incubation time was determined in order to generate the curves of uncorrected disappearance, corrected with the ^{35}S, cytosine and by the ND method (Mehrez and Orskov, 1977). The parameters produced from these curves were used to generate the respective RDP values at 0.05 /h rumen outflow rate.

The RDPs were subjected to a two-way analysis of variance (ANOVA) with method of correction as the treatment and feed as the block. The Student Newman-Keuls (SNK) test was used to separate the means due to the method of correction.

Results

Chemical composition of experimental feeds

The chemical fractions of the feeds incubated in the rumen are presented in Table 1. The N contents of the diets were low and the soluble portion of the crude protein content was higher in SBP than in hay. The NDF and ADF contents of hay were higher than those of SBP with the ADF content of hay being about two times that of SBP.

In situ protein degradability

The calibration curve for cytosine is shown in Figure 1. The slope of the curve measured 1.09 and the intercept was 0.01. The r2 value was 1. In decreasing order of magnitude the RDPs of hay were as corrected by cytosine (0.82), 35S (0.75) and ND (0.74), with the cytosine figures being statistically higher than the rest, and the 35S and ND values also being substantially higher ($P<0.05$) than the uncorrected (0.62) (Table 2, Figures 2a and 2b). For SBP, the RDPs as corrected by the methods were considerably different ($P<0.05$), with the RDP corrected with cytosine (0.84) being the highest followed by the 35S (0.81), then the ND (0.76) method before the uncorrected (0.73).

There was a significant interaction ($P<0.05$) between the method of correction of microbial contamination and the type of feed. No differences were observed between the sheep concerning N loss from the bags. The extent of microbial contamination of hay (24.6%) residue appeared to be greater than that of SBP (9.5%). Nitrogen loss from bags after mere washing was higher in SBP (0.60) compared to hay (0.35) (Table 3).

Discussion

In situ degradability

The ^{35}S technique was, comparatively, the most laborious and suggestively the least precise of the three methods, followed by the cytosine technique and then the ND method. However, the ^{35}S procedure appeared to be credible because it is thought to mark microbes only, distinguishing them from feed. The cytosine contents of the residues especially

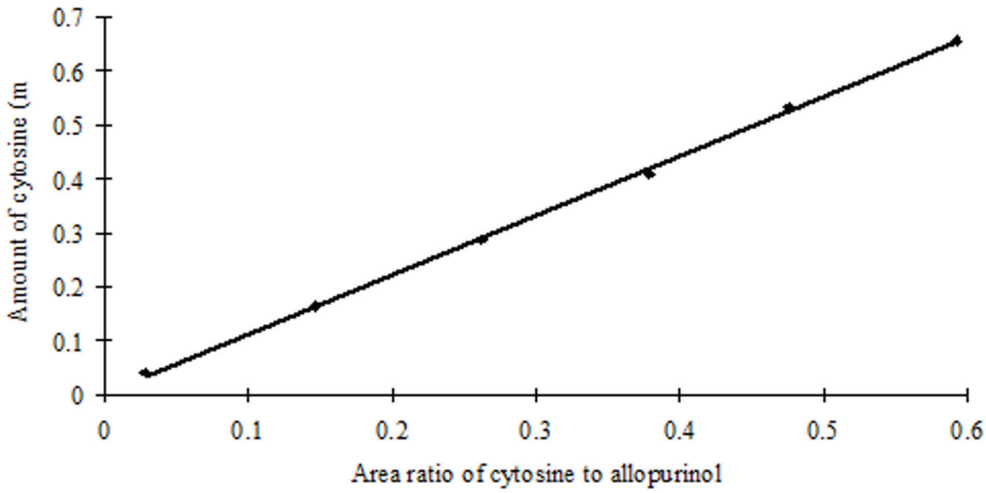

Figure 1: Cytosine multi-level calibration curve (heating applied to samples). The equation was y = 1.09(s.e.=0.015)*** x + 0.01(s.e.=0.005)NS; P<0.001; r2 = 1.00; MSE=0.015; n= 6. The terms: y, amount of cytosine (mg); x, area ratio of cytosine to allopurinol; s.e., standard error of estimate; ***, significant at P<0.001; NS, not significant; MSE, mean square error.

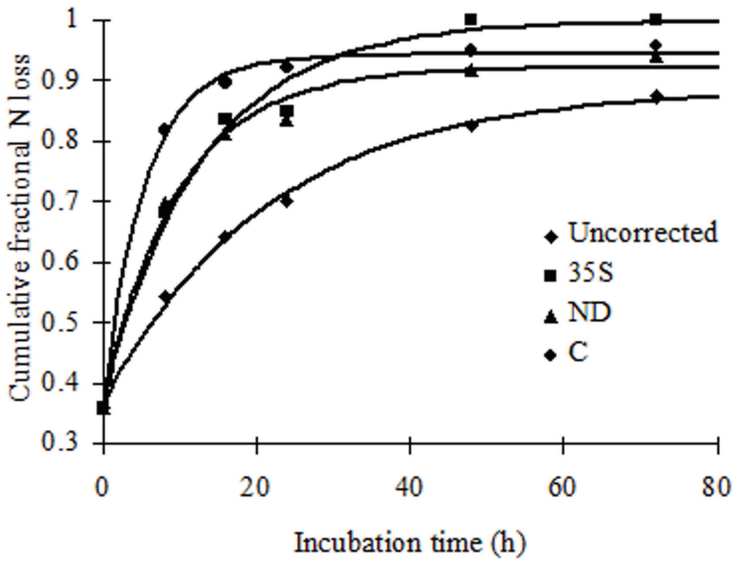

Figure 2a: In situ fractional protein degradation of hay uncorrected and corrected for microbial contamination using the marker/N ratios of the solid associated microbes or the neutral detergent fibre method.

at the shorter times of incubation were likely to be of both microbial and feed sources. At zero h of incubation, rumen microbial matter in feed residue is assumed to be non-existent. Cytosine found in feed at this hour of incubation would be feed cytosine. Avornyo (1999) has indicated high levels of cytosine at 0 h incubation. The use of residual cytosine to estimate contamination of the residue with microbes, without accounting for residual content of feed cytosine can lead to substantial overestimation of corrected N disappearance from incubated feeds. Perez et al. (1996) have used 15N enrichment of ruminal matter to partition residual purine bases into that of microbial origin and the fraction contributed

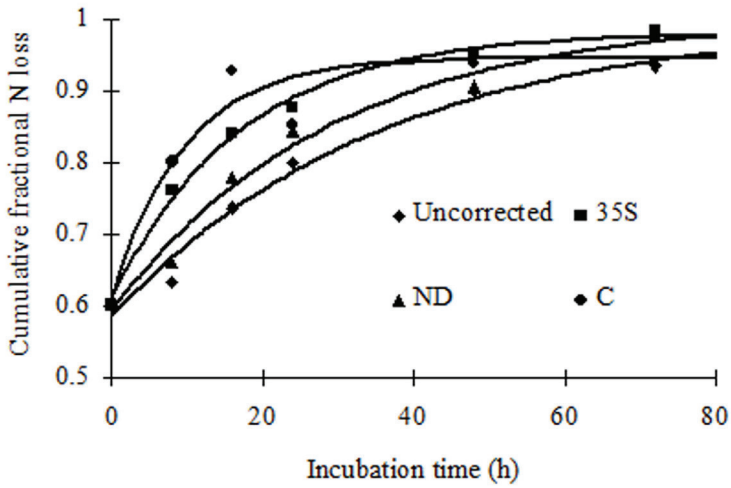

Figure 2b: In situ fractional protein degradation of sugarbeet pulp uncorrected and corrected for microbial contamination using the marker/N ratios of the solid associated microbes or the neutral detergent fibre method

by feed material, and to determine the rate of feed purine base degradation. Machine-washed zero h incubation feed residues when subjected to ^{35}S counting gave figures higher than the background values (Table 3). This result was unexpected and so an experiment was undertaken (Avornyo, 1999) to verify if there had been inadvertent contamination of the zero h residues with ^{35}S. The result suggested greater emission of low energy photons from 0 to 6 keV from the samples containing hay and SBP than that containing distilled water (Figure 3). The differences in the emissions observed at the 0 to 6 lower limits might be because of luminescence events in the scintillation vials, which can be eliminated if the luminescence option is installed in the liquid scintillation system (Liquid Scintillation Systems Operation Manual, 1983). Alternatively, vials for counting could be rested in a dark place for 1 h to minimize excitation of the scintillation solution. The ND technique solubilizes cell content including the protein. When a sample of SAM was boiled in NDS, about 0.975 of the microbial protein was solubilized (Avornyo, 1999). This observation was extended to micro-organisms adhered to bag residues. At zero h incubation, when no microbial protein was assumed present, ND procedure solubilized protein from the residues equal to the protein that was insoluble in water but soluble by the NDF method. This protein fraction is called protein B2 by the Cornell method of protein degradation (Sniffen *et al.,* 1992; Lanzas *et al.,* 2008). The rate of degradation of feed protein B2 is estimated to range from 0.05 to 0.15 h-1. Therefore, the amount of feed protein B2 in residues retrieved from in situ bags could be substantial for incubation times ranging from 0 to 16 h. An adjustment of the corrected N loss by the ND method was made (Sniffen *et al.,* 1992; Avornyo, 1999) by accounting for the portion of the feed residue protein B2 that was dissolved by the ND method and subtracting it from the measured N loss corrected by the ND method. Because the ND method did not appear to dissolve all the microbial protein, it might be underestimating microbial contamination. However, because the method removed feed protein B2 from the early incubation time bags, this could counteract the underestimation. The underestimation would be expected to be higher at later incubation stages when there would be virtually no protein fraction B2 left. Moreover, the proportion of microbial protein in residue would increase as period of incubation extends.

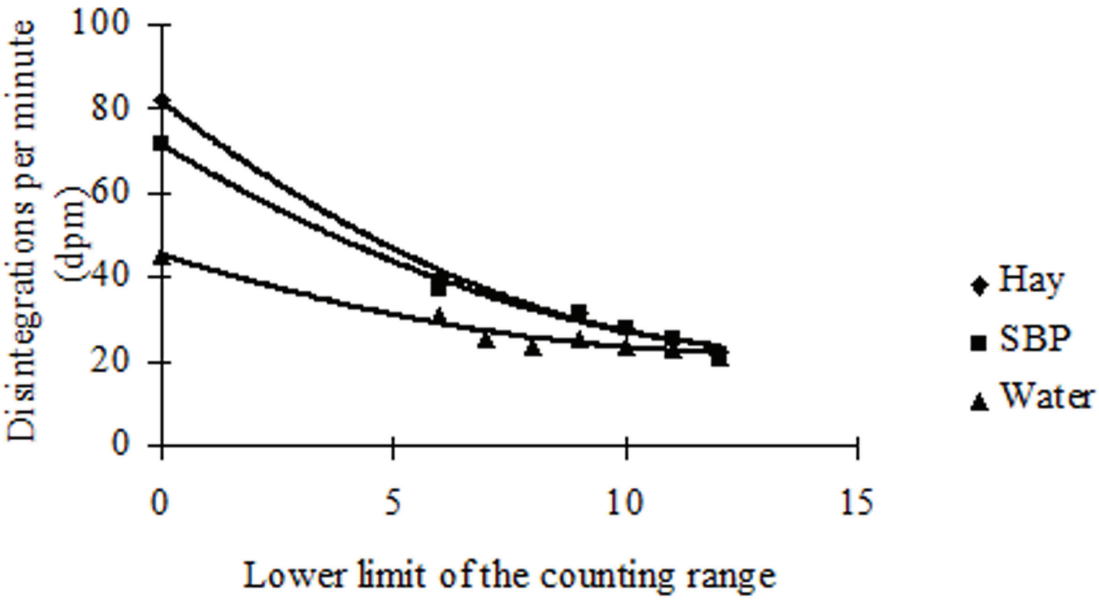

Figure 3: The disintegrations per minute values of zero h hay residue, zero h sugarbeet pulp residue and distilled water at different lower limits of the counting range of a scintillation counter

Table 1: Chemical composition† (fraction of dry matter) of the studied feeds.

Feeds	OM	N	NDF	ADF	SP	SP/(CP)
Hay	0.912	0.034	0.620	0.239	0.058	0.272
±SBP	0.901	0.020	0.399	0.116	0.057	0.465

† OM, organic matter; N, nitrogen; NDF, neutral detergent fibre; ADF, acid detergent fibre; SP, soluble protein; CP, crude protein; ‡SBP, sugarbeet pulp

Table 2: The constants used to describe in situ protein degradation and estimated rumen degradable protein (± s.e.m).

Feed	Constant	Apparent	Actual as estimated using:		
			^{35}S	ND	Cytosine
Hay	†a	0.37	0.37	0.37	0.36
	b	0.52	0.63	0.55	0.58
	c	0.05	0.08	0.10	0.18
	*RDP (kp=0.05/h)	0.62^{c}± 0.01	0.75^{b}± 0.02	0.74^{b}± 0.01	0.82^{a} ± 0.01
SBP	†a	0.59	0.61	0.59	0.61
	b	0.41	0.37	0.41	0.34
	c	0.03	0.06	0.04	0.11
	*RDP (kp=0.05/h)	0.73^{d}± 0.01	0.81^{b} ± 0.01	0.76^{c} ± 0.01	0.84^{a} ± 0.00

* RDP = rumen degradable protein; s.e.m., standard error of the mean; †the zero h values were obtained by the machine washing only of feed in in situ bags. Values with different superscript letters in a row are significantly different. Correction with marker/N ratio made use of the solid associated microbes.

Table 3: Apparent microbial contamination of zero hour incubation nitrogen disappearance values (± s.e.m.) obtained after the application of the methods of correction to the zero hour values.

Feed	Observed 0 h	Estimated 0 h after correction with:		
		^{35}S	ND	Cytosine
Hay	0.35 ± 0.015	0.39 ± 0.012	0.58 ± 0.005	0.74 ± 0.044
Sugarbeet pulp	0.60 ± 0.023	0.63 ± 0.024	0.59 ± 0.003	0.73 ± 0.073

s.e.m., standard error of the mean; n = 6. Correction with the marker/N ratios made use of the solid associated microbes.

Conclusion

In the absence of reliable methods or simple techniques to account for the loss of feed protein fraction B2 and dietary cytosine, the use of the 35S procedure was considered to be the most appropriate, although not necessarily the most precise method for estimating in situ actual N loss from bags incubated in the rumen.

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COMPARISON OF IN SITU AND CORNELL METHODS OF ESTIMATING RUMEN DEGRADABLE PROTEIN OF RUMINANT FEEDSTUFFS

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Abstract

The objective of this experiment was to compare in situ and Cornell methods of estimating the rumen degradable protein (RDP) of a variety of ruminant feedstuffs. Samples of barley meal, soyabean meal, fishmeal, linseed meal, maltculms, maize gluten meal, double zero rapeseed meal, high glucosinolate rapeseed meal, sunflower meal and field beans were used for the Cornell in vitro technique. The same concentrate samples were analyzed by Mirza (1993) for in situ protein degradation. Regression analysis was used to relate the Cornell RDP values to corresponding in situ values. The Cornell RDP estimates averaged 0.10 less than the in situ values. However, the Cornell RDP estimates were significantly correlated with those of the in situ method for the same feeds (r2 0.76; P<0.001). Comparison of other in situ and Cornell data on nominally similar feedstuffs showed some level of agreement in the RDP estimates especially of concentrate and by-product feedstuffs. The Cornell model therefore compares favourably with the in situ method for estimating RDP of concentrate and by-product feedstuffs. There were however differences in the magnitude of the RDP values with the in situ method generally recording higher values.

Keywords: Rumen, concentrate, feeds

COMPARISON DES METHODES IN SITU ET CORNELL POUR L'ESTIMATION DE LA DEGRADATION DANS LE RUMEN DE PROTEINES CONTENUES DANS LES ALIMENTS DE RUMINANTS

Résumé

Cette expérience avait pour objectif de comparer les méthodes in situ et Cornell pour l'estimation de la dégradation dans le rumen de protéines contenues dans une variété d'aliments des ruminants. Des échantillons de farine d'orge, de farine de soja, de farine de poisson, de tourteau de lin, de tiges de malt, de farine de gluten de maïs, de tourteau de colza double zéro, de tourteaux de colza à haute teneur en glucosinolate, de tourteau de tournesol et des féveroles ont été utilisés pour la technique in vitro Cornell. Les mêmes échantillons de concentrés ont été analysés avec la technique décrite par Mirza (1993) pour la dégradation des protéines in situ. L'analyse de régression a été utilisée pour comparer les valeurs Cornell de dégradation des protéines dans le rumen (RDP) aux valeurs in situ correspondantes. Les estimations RDP par la technique Cornell étaient en moyenne de 0,10 moins que les valeurs in situ. Cependant, il y avait une corrélation significative entre les estimations RDP par la méthode Cornell et celles de la méthode in situ pour les mêmes aliments (r2 0,76; P <0,001). La comparaison des autres données des méthodes in situ et Cornell relatives aux aliments théoriquement similaires ont montré un certain niveau de concordance dans les estimations RDP, en particulier celles des concentrés et des sous-produits alimentaires. Le modèle de Cornell concorde favorablement avec la méthode in situ pour l'estimation des RDP des concentrés et sous-produits alimentaires. Cependant, des différences ont été notées dans la grandeur des valeurs RDP, la méthode in situ enregistrant généralement des valeurs plus élevées.

Mots-clés: Rumen, Concentré, Aliments

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Introduction

The in situ method has been the most widely accepted alternative to the in vivo for estimating the degradation of proteins. Neither is practical for commercial laboratories but can be tools for generating results for use in developing in vitro methods (Roe *et al.,* 1991). A desired in vitro technique will be one that employs a simple physical or chemical laboratory method to describe degradation properties (Broderick *et al.,* 1991). Notable amongst in vitro techniques are the solubility, enzymatic, continuous culture and the Cornell model for protein fractionation. Results have indicated poor to variable prediction of in situ data by the solubility (Shannak *et al.,* 2000; Mathis *et al.,* 2001), enzymatic and continuous culture studies. The Cornell Net Protein System uses chemical solutions to separate feed protein into five fractions that are thought to have variable rumen degradabilities owing to their different rates of degradation. Krishnamoorthy *et al.* (1982) summarized the Cornell protein fractionation procedure as fast, low cost and ideal for regular evaluation of feedstuffs. There is, however, limited information on the predictability of in situ protein degradation by the Cornell estimates. For instance in their review of existing methods to study rumen degradation, Mohamed and Chaudhry (2008) made no mention of the Cornell method.

The objective of the study was to compare the in situ and the Cornell methods of estimating the protein degradabilities of a variety of ruminant concentrate feedstuffs.

Materials and Methods

Feed protein fractionation

Samples of barley meal (BM), soyabean meal (SBM), fishmeal (FM), linseed meal (LM), maltculms (MC), maize gluten meal (MGM), double zero rapeseed meal (00-RSM), high glucosinolate rapeseed meal (HG-RSM), sunflower meal (SFM) and field beans (FB), milled to 1 mm particle size, were used for the Cornell in vitro technique. The same concentrate samples were analyzed by Mirza (1993) for in situ protein degradation. The recommended Cornell method proposed by Licitra *et al.* (1996) was followed. The rates of degradation of the protein fractions determined in this experiment were taken from the Cornell databank. A common rate of passage of feed of 0.05/h was adopted.

The in situ experiment

This was based on the method of ARC (1984). It involved incubating 5 g air-dry samples, in series, in the rumen of wethers. Residual nitrogen (N), after incubation, was determined by the Kjeldahl analysis (Mirza and Miller, 2005). The exponential equation of Mehrez and Orskov (1977) was used to describe the degradation, and to obtain the water-soluble protein (a), the insoluble but potentially available rumen protein (b) and the in situ degradation rate constant (c) values, and also the rumen degradable protein (RDP) at a specified rumen outflow rate.

Statistical analysis

Regression analysis was used to relate the Cornell values to corresponding in situ values. Paired T-test was used to compare means due to method of protein fraction. Minitab version 10 package was also used to determine outliers in datasets.

Results

Protein fractions

Dry matter and protein fractions of the ten concentrate feedstuffs are presented in Tables 1a and 1b. The crude protein (CP) content of the concentrates ranged from 0.13 to 0.72 of dry matter (DM). Generally, the concentrates contained less than 0.50 buffer-soluble N (fraction of CP).

The borate phosphate (BP) buffer soluble protein (SP) is compared with in situ water-soluble fraction "a", and the pooled degradation rate of fractions B2 (Cornell true protein of intermediate degradability) and B3 (Cornell true protein of slow degradability), kdB2+3 , compared with in situ degradability rate constant "c" in Table 2. A good agreement is observed between the "a" values and BP buffer SP (r2 0.73; $P<0.01$). Even though mean SP appeared higher than mean "a", the difference was not statistically significant (Table 2).

Comparison of the in situ protein degradation rate (c) with the pooled degradation rate for fractions B2 and B3, using regression analysis, showed significance (r2 0.42; P<0.05). The "c" values on average were significantly higher (P<0.05) than the pooled degradation rate obtained for B2 and B3.

Degradability

Extraction of non-protein nitrogen (NPN) with tungstic acid (TA) or with trichloroacetic acid (TCA) for subsequent estimation of rumen degradable protein (RDP) was assessed. The use of TA or TCA produced similar RDP values. Trichloroacetic acid-RDP was, on average, 0.002 greater than TA-RDP, for the feeds studied. A regression analysis of TCA-RDP on TA-RDP was significant (r2 1.00; P<0.001). Similarly, in the estimation of RDP, A and B1 pools kept separately and assigned different degradation rates produced a slightly lower RDP value - about 0.001 less - than when A and B1 fractions were pooled and assigned infinite rate of degradation.

Table 3 shows RDP estimated by the Cornell method and by the in situ method done by Mirza (1993). For most of the concentrates, there was a good agreement between the Cornell values and those determined by the in situ method. However, the Cornell values were lower than the in situ values except for MGM. The Cornell estimates averaged 0.10 less RDP than the in situ values (P<0.05).

Other datasets were analyzed:

a) Alderman and Cottrill's (1993) list of in situ protein fractions ("a", "b" and "c") of some feedstuffs were used to calculate RDP at 0.05/h rumen outflow rate. These values were then compared with calculated Cornell RDPs for nominally similar feedstuffs from Mansbridge (1996) list of Cornell protein fractions (A, B1, B2, B3 and C) of these feedstuffs. Thirty three feedstuffs were compared, in total. When all the 33 nominally similar feedstuffs consisting of concentrates, forages and by-product feedstuffs were compared, the regression equation was statistically significant (P<0.001) (Figure 1). When the forages in this set of 33 samples were removed and analyzed separately, they were not related (Figure 2). However the mean RDP was 0.82 for both the Cornell from Mansbridge (1996) and in situ from Alderman and Cottrill (1993), respectively. Excluding the forages from the set of 33 samples, the relationship for the remaining samples was still significant even though the coefficient of determination (r2) was numerically lower and level of significance had changed from P<0.001 to P<0.01 (Figure 3).

b) Mansbridge (1996) also reported on in situ and Cornell protein fractions of another set of 15 feeds. Two of the feeds were not included in this analysis because there was inadequate information on them. The remaining feeds, consisting of concentrates and forages were analyzed separately for in situ RDP and Cornell RDP, at 0.05/h rumen outflow rate. The relationship between them was not significant (Figure 4). The in situ "a" value for one of the feeds; distillers' dark grains (wheat), was over 0.81 compared to 0.35 SP obtained with the Cornell method, for the same feed. De Boever et al. (1997) excluded brewers' grains from their data because it disturbed their regression equations, and cited Cone et al. (1995) as having faced similar difficulties with brewers' grains. When the RDP value of distillers' dark grains was removed from the dataset, the relationship became statistically significant (Figure 5). When the RDP values of rapeseed meal and one silage were also removed because Minitab version 10 analysis showed them to be outliers, the regression equation was further improved; r2 was 0.91 and P<0.001. The RDP mean values were 0.80 and 0.79 for the in situ and Cornell, respectively.

Discussion

Neutral detergent insoluble protein was generally more than ADIP in the concentrate feedstuffs studied. However, the difference between NDIP and ADIP in SBM and SFM was small. Krishnamoorthy *et al* (1982) found ADIP to exceed NDIP in MGM and SBM, which they attributed to the presence

of Maillard products in their samples. Maillard products tend to be insoluble in acid detergent solution (ADS) but soluble in neutral detergent solution (NDS) (Krishnamoorthy *et al.,* 1982). They are also insoluble in BP buffer. Hence with the Cornell chemical method, Maillard products even though they may be only partially digestible, are accounted for two times; one, as part of ADIP and two, as fraction B2. Maillard products will be accounted for only once, as part of B2, if neutral detergent analysis of a sample is followed by acid detergent analysis on the same sample, for the determination of ADIP. The amount of Maillard products in a feed sample may be determined by performing acid detergent analysis on the sample followed by neutral detergent analysis on the same sample.

The Cornell estimates of RDP in the present experiment were highly correlated with the in situ RDP estimated by Mirza (1993) for the same feeds. This indicates that there is some agreement between the Cornell method and the in situ method for estimation of RDP (Shannak *et al.,* 2000). However, the estimated Cornell RDPs in the present study were lower than estimated by Mirza (1993). One reason for this observation is the higher rate of degradation ("c") of the insoluble protein measured by Mirza (1993), compared to the degradation rates suggested in the Cornell

Table 1a: Dry matter and protein fractions (± s.e.m. of n = 3 for protein fractions and n = 2 for dry matter) of the concentrate feedstuffs

Feed	DM, fra. of as is	CP, fr. of DM	SPb, fr. of CP
Barley meal	0.87 ± 0.002	0.13 ± 0.001	0.24 ± 0.002
Soyabean meal	0.88 ± 0.000	0.54 ± 0.005	0.18 ± 0.009
Fish meal	0.92 ± 0.001	0.72 ± 0.003	0.21 ± 0.005
Linseed meal	0.89 ± 0.000	0.40 ± 0.004	0.46 ± 0.007
Malt culms	0.93 ± 0.001	0.33 ± 0.001	0.44 ± 0.014
Maize gluten meal	0.88 ± 0.001	0.71 ± 0.004	0.03 ± 0.002
00-Rapeseed meal	0.89 ± 0.000	0.38 ± 0.003	0.19 ± 0.006
HG-Rapeseed meal	0.88 ± 0.000	0.38 ± 0.001	0.26 ± 0.004
Sunflower meal	0.88 ± 0.000	0.35 ± 0.003	0.46 ± 0.016
Field beans	0.88 ± 0.002	0.26 ± 0.003	0.66 ± 0.005

afr., fraction.
bSP, soluble protein.

Table 1b: Protein fractions (± s.e.m. of n = 3) of the concentrate feedstuffs

Feed	NPN[a], fr[b]. of SP	NDIP[c], fr. of CP	ADIP[d], fr. of CP
Barley meal	0.36 ± 0.085	0.19 ± 0.015	0.03 ± 0.003
Soyabean meal	0.04 ± 0.066	0.05 ± 0.014	0.05 ± 0.004
Fish meal	0.05 ± 0.049	0.47 ± 0.013	0.01 ± 0.001
Linseed meal	0.004 ± 0.016	0.20 ± 0.045	0.03 ± 0.001
Malt culms	0.69 ± 0.030	0.39 ± 0.007	0.02 ± 0.001
Maize gluten meal	0.38 ± 0.133	0.20 ± 0.010	0.12 ± 0.004
00-Rapeseed meal	0.22 ± 0.019	0.14 ± 0.011	0.06 ± 0.002
HG-Rapeseed meal	0.22 ± 0.026	0.14 ± 0.022	0.06 ± 0.002
Sunflower meal	0.13 ± 0.026	0.10 ± 0.013	0.07 ± 0.006
Fvield beans	0.12 ± 0.018	0.17 ± 0.003	0.03 ± 0.001

aNPN is expressed as NPN*6.25
fr., fraction.
cNDIP, neutral detergent insoluble protein
dADIP, acid detergent insoluble protein

Table 2: Comparison of Mirza (1993) in situ protein degradability parameters with those of the Cornell method for the same set of concentrate feedstuffs. Values are presented as fractions of total crude protein except "c" and kdB2+3 which are given as fractions per hour

Feed	"a"	SP	"c"	k_{dB2+3}
Barley meal	0.12	0.24	0.32	0.08
Soyabean meal	0.02	0.18	0.11	0.11
Fish meal	0.34	0.21	0.02	0.01
Linseed meal	0.33	0.46	0.08	0.04
Maltculms	0.49	0.44	0.08	0.01
Maize gluten meal	0.08	0.03	0.04	0.04
00-Rapeseed meal	0.26	0.19	0.13	0.09
HG-Rapeseed meal	0.24	0.26	0.16	0.09
Sunflower meal	0.36	0.46	0.21	0.10
Field beans	0.60	0.66	0.13	0.05
Mean	0.28	0.31	0.13	0.06
s.e.m.; P-value			0.022; P=0.38	0.016; P<0.05
r^2; P-value			0.73; P<0.01	0.42; P<0.05

Table 3: Estimation of rumen degradable protein (RDP) in the rumen (fraction of total crude protein)

Feed	Cornell method	In situ method
Barley meal	0.62	0.78
Soyabean meal	0.71	0.74
Fish meal	0.32	0.45
Linseed meal	0.65	0.72
Maltculms	0.52	0.78
Maize gluten meal	0.37	0.22
00-Rapeseed meal	0.64	0.78
HG-Rapeseed meal	0.66	0.80
Sunflower meal	0.75	0.86
Field beans	0.76	0.91
r^2; P-value		0.76; P<0.01

databank for similar protein fractions. It is expected that in situ RDP values will decrease upon correction for fine particle loss from in situ bags during incubation. One method of correction for fine particle loss, as proved by Weisbjerg *et al.* (1990), may cause a change of the in situ "a", "b" and RDP but not the "c" value. Shannak et al. (2000) showed that the agreement between the Cornell method and in situ method improved if a higher rumen solid outflow rate of 0.08/h was adopted, which is consistent with high feed intake and productivity of ruminants. In spite of this there still appears to be a slight under-prediction bias of the Cornell method on the performance of both growing cattle and lactating cows (Fox *et al.,* 2004). Gosselink *et al.* (2004) observed that compared to the in situ technique there was a marked departure of the Cornell method in predicting in vivo rumen escape nitrogen. In the updated version of the Dutch protein evaluation system for ruminants, Van Duinkerken *et al.* (2010) were of the opinion that the Cornell method in its present form is not very accurate.

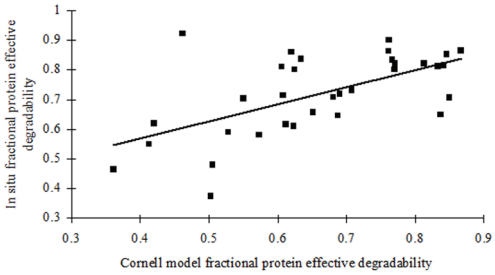

Figure 1: The relationship between in situ and the Cornell protein degradability of nominal classes of ruminant feeds (In situ data from Alderman and Cottrill (1993) and Cornell data from Mansbridge (1996)).The equation was in situ = 0.58(s.e.=0.14)*** × Cornell + 0.34(s.e.=0.09)**; P<0.001; r2 = 0.36; MSE=0.11; n = 33. The terms: s.e., standard error of estimate; **, significant at P<0.01; ***, significant at P<0.001; MSE, mean square error.

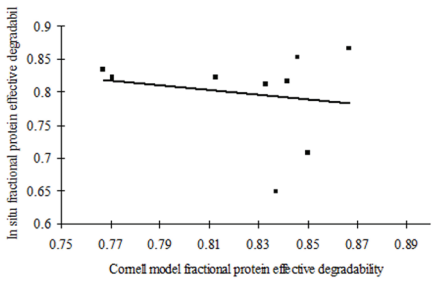

Figure 2: The relationship between the in situ and the Cornell protein degradability of nine nominal classes of forages (In situ from Alderman and Cottrill (1993) and Cornell data from Mansbridge (unpublished data)). The equation was in situ = -0.34(s.e.=0.76)NS × Cornell + 1.08(s.e.=0.63) NS; P=0.67; r 2 = 0.03; MSE = 0.08; n = 9. The terms: s.e., standard error of estimate; NS, not significant; MSE, mean square error.

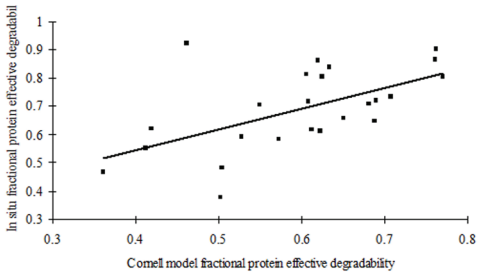

Figure 3: The relationship between in situ and the Cornell protein degradability of nominal classes of ruminant feeds excluding the forages. In situ data from Alderman and Cottrill (1993) and Cornell data from Mansbridge (unpublished data). The equation was in situ = 0.74(s.e.=0.22)** × Cornell + 0.25(s.e.=0.13)NS; P<0.01; r2 = 0.33; MSE=0.12; n = 24. The terms: s.e., standard error of estimate; NS, not significant; **, significant at P<0.01; MSE, mean square error.

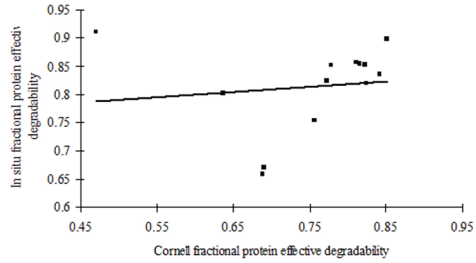

Figure 4: Prediction of in situ protein degradability by the Cornell method for 13 feeds, using chemical and in situ data on the same feed samples reported by Mansbridge (unpublished data). The equation was in situ = 0.09(s.e.=0.22)NS × Cornell + 0.75(s.e.=0.16)***; P=0.68; r 2 = 0.02; MSE = 0.08; n = 13. The terms: s.e., standard error of estimate; NS, not significant; ***, significant at P<0.001; MSE, mean square error.

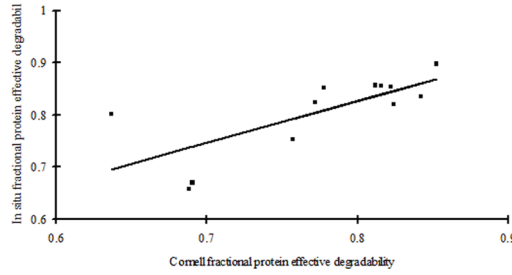

Figure 5: A regression plot between in situ and the Cornell protein degradabilities on 12 (excluding distillers' dark grains) of the 13 feed samples reported by Mansbridge (unpublished data). The equation was in situ = 0.80(s.e.=0.23)** × Cornell + 0.19(s.e.=0.18)NS; P<0.01; r2 = 0.54; MSE = 0.05; n = 12. The terms: s.e., standard error of estimate; NS, not significant; **, significant at P<0.01; MSE, mean square error.

The difference in the RDP values for MGM (0.22 RDP by Mirza (1993) and 0.37 RDP by the Cornell method in the present study) was unusual and might be attributed to using too short a time in the in situ estimation to get the degradability end point. Maize gluten meal, being a glutenous substance might cause clogging of bag pores preventing flux of material to and from the bag. It could also be that MGM contained significant amounts of Maillard

products which would increase RDP estimated by the Cornell method, since Maillard products are also included in B2 and therefore assigned intermediate degradability.

The lack of agreement between in situ (Alderman and Cottrill (1993)) and the Cornell (Mansbridge (1996)) RDP for the forages might be due to the narrow range of their RDP values, and to the fact that nominally similar rather than the same forages were compared. Also, the in situ values for forages might have been affected by microbial contamination of bag residues, which reduced the apparent RDP values. Variation between laboratories in the measurement of protein degradability has also been associated with differences in the type of bag material and the methods of sample preparation and estimation of crude protein (CP) (Stern *et al.,* 1997).

The lack of significant relationship between the Cornell analysis and in situ estimates of RDP with the 13 samples of Mansbridge (1996) appeared to be partly the result of loss of heat damaged protein from in situ bags, some of which the Cornell procedure recovered as insoluble protein.

Conclusion

The Cornell model for RDP was able to predict in situ method for RDP for the same as well as similar concentrate and by-product feedstuffs. There were however differences in the magnitude of the RDP values with the in situ method generally recording higher values. The Cornell method however did not predict in situ RDP for forages. This appears to be partly due to microbial contamination of feed residues in in situ bags, and also to the narrow range of the RDP values as well as the fact that nominally similar rather than the same forages were compared.

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BROWSING CAPACITY AND NUTRITIVE VALUE OF INDIGENOUS BROWSES IN A TROPICAL COASTAL SAVANNAH RANGELAND

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Abstract

The study sought to identify indigenous browse species in the Coastal Savannah of Ghana, evaluate their browsing capacity and nutritive value during the dry season. It was hypothesized that indigenous browses maintain a high nutritive value and contribute immensely to livestock feed in the dry season. Data were collected within a 2 m band along a 100 m long transect in thirteen randomly selected sites. Data included; name, plant height, canopy height and canopy radius of browses. Indigenous browses identified were; *Securinega virosa, Zanthoxylum xanthoxyloides, Flacourtia flavescens, Capparis erythrocarpos, Diospyros abyssinica, Mellittia thonningii, Urena lobata and Dichrostachys glomerata.* Crude protein (CP) contents of browse leaves ranged from 85.4 (*Diospyros abyssinica*) to 161.2 g/kg (*Urena lobata*), neutral detergent fibre (NDF) ranged from 244.0 (*Securinega virosa*) to 488.2 g/kg (*Dichrostachys glomerata*), acid detergent fibre (ADF) content ranged from 121.0 (*Securinega virosa*) to 329.8 g/kg (*Dichrostachys glomerata*) all on dry matter (DM) basis. The browses had an average browse unit of 447.1 per hectare and a browsing capacity of 1 tropical livestock unit (TLU) per 1.4 hectares per month. It was concluded that indigenous browses maintain a high nutritive value in the dry season and have a huge potential in curbing the dry season feed deficit.

Keywords: Indigenous browse species, Browsing capacity, Nutritive value, Coastal Savannah

CHARGE LIMITE ETVALEUR NUTRITIVE DES ESPÈCES LIGNEUSES INDIGÈNES DANS UN PARCOURS DE LA SAVANE CÔTIÈRE TROPICALE

Résumé

L'objectif de l'étude était d'identifier les espèces ligneuses indigènes dans la savane côtière du Ghana, d'évaluer leur charge limite et leur valeur nutritive pendant la saison sèche. L'hypothèse de départ était que les espèces ligneuses indigènes gardent une valeur nutritive élevée et contribuent énormément à l'alimentation du bétail pendant la saison sèche. Les données ont été recueillies sur une bande de 2 m de largeur et 100 m de longueur dans treize sites choisis de manière aléatoire. Les données comprenaient le nom, la hauteur des plantes, la hauteur et le rayon du couvert végétal. Les espèces ligneuses indigènes identifiées étaient :*Securinega virosa, Zanthoxylum xanthoxyloides, Flacourtia flavescens, Capparis erythrocarpos, Diospyros abyssinica, Mellittia thonningii, Urena lobata* et *Dichrostachys glomerata*. La teneur en protéines brutes (CP) des feuilles de ces espèces variait entre 85,4 (*Diospyros abyssinica*) et 161,2 g / kg (*Urena lobata*) ;la teneur en fibreau détergent neutre (NDF) variait entre 244,0 (*Securinega virosa*) et 488,2 g / kg (Dichrostachys glomerata), et la teneur en fibre au détergent acide (ADF) variait entre 121,0 (*Securinega virosa*) et 329,8 g / kg (*Dichrostachys glomerata*), mesurées toutes sur matière sèche (MS). Les espèces ligneuses avaient une unité de paissance moyenne de 447,1 par hectare et une charge limite d'une (1) unité de bétail tropical (TLU) par 1,4 hectare par mois. Il a été conclu que les espèces ligneusesindigènesgardentune valeur nutritive élevée pendant la saison sèche et ont un potentiel énorme de réduction du déficit alimentaire pendant la saison sèche.

Mots-clés: Espèces ligneuses indigènes, Charge limite, Valeur nutritive, Savane côtière

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Introduction

Woody shrub and tree species are important components of tropical savannah rangelands. These woody components consist of both browse and non-browse species. Browses constitute very important component of livestock feeding. Anugwa *et al.* (2000) reported that, the rate of fluctuation in the nutritive value of browses with changing seasons is less drastic than that of grasses. Other attributes of browse species are their perenniality, provision of high protein quality for livestock and ease to maintain (Gutteridge and Shelton, 1994). Browses contain high levels of digestible protein, minerals and vitamins, which can play major roles in improving intake of roughage by ruminants (Bayer, 1990; Larbi *et al.,* 1993). Kennedy *et al.* (2002) reported an increased ruminant utilization of tropical dry season grass by feeding them on fallen browse leaves of *Albizia lebbek*.

Despite the important role browses play in livestock feeding, most studies in Ghana on browses have focused mostly on leaf quality (Sottie *et al.,* 1998; Annan and Tuah, 1999; Oddoye *et al.,* 2005) and livestock performance on them (Annan and Tuah, 1999; Oddoye *et al.,* 2005) for just a selected preferred browse species. There is currently no report on the browsing capacity in the Coastal Savannah rangeland of Ghana. An inventory of indigenous browse species has also not been made in recent times. Peyre de Fabregues (1975) observed that, many tropical range ecologists have not studied in any detail the contribution of browse to rangeland nutrition because of the difficulties in quantifying browse components. In recent times however, improved methodologies and equipment have been developed to facilitate the estimation of rangeland productivity and browse capacity. Yet, there is still scarcity of data on Ghanaian Savannah rangelands. Conceivably, an inventory of indigenous browses, their nutritive value and an evaluation of the browsing capacity of the Coastal Savannah rangeland of Ghana will contribute immensely to providing information on total forage supply (grass and browse) for effective livestock production on one hand and for efficient management of browses on the other hand for sustainable use of available range resources. The objectives of this study were to; i) identify indigenous browse species ii) evaluate their nutritive value and estimate the browsing capacity in the dry period of the Coastal Savannah agro-ecological zone.

It was hypothesized that indigenous browse species in the Coastal Savannah rangelands of Ghana maintain a high nutritive value and thus, contribute immensely to livestock feeding in the dry season.

Materials and Methods

The Study Area

The Coastal Savannah rangelands of Ghana lies between latitude 40 55'N and 60 05' N and longitude 10 45' W and 00 30' E of the meridian. It is divided into two broad sections; the south-eastern plains, east of the capital Accra and the south-western plains, west of Accra. The rainfall regime of this area is the dry equatorial type with the mean annual rainfall ranging between 600 and 1000 mm (Alhassan *et al.,* 1999). The major rainy season occur in the months of May to July and the minor in September to October (Rose-Innes, 1977). The long dry season in this agro-ecological zone starts in November and ends mostly in March. The major soils include; the Coastal Savannah ochrosols, the lateritic sandy soils, the tropical black clays (Vertisols), the tropical grey earths (Alfisols), the sodium vleisols and the coastal sands (Benneh *et al.,* 1990).

Data collection

Thirteen randomly selected sites in Dangme East and Dangme West districts were thoroughly studied. These sites were grazing areas representative of the vegetation cover of the area and without visible signs of any recent human disturbances in the form, wood harvesting (tree stumps) and burning (burnt parches and burnt tree stems). In each site, a woody plant was used as a reference point and a transect of 100 m was laid along a randomly selected direction. Pegs were placed on both sides of the 100 m transect at a distance of 1 m from the transect line. Data were collected on all browse species found within the 2 m band along the length of the transect. Data collected

were the name, plant height (measured from the ground to the highest point using a measuring staff), canopy height (height from the ground to the base of the canopy) and the horizontal canopy radius (length from the stem to canopy perimeter) of all browse species. Due to the unsymmetrical nature of the woody browse species, canopy radius was measured twice at different directions and the average of these two measurements recorded as the canopy radius for that particular browse plant. The palatability of browse species was ascertained using expert and indigenous knowledge.

Estimation of mean canopy volume

To evaluate the standard mean canopy volume for browse species in the study area, measurements were made on several browse species with whole plant height between 1.48 – 1.52 m tall. Their canopy volumes were calculated and the mean served as the mean canopy volume for all the browse species. The mean canopy volume was subsequently used to calculate the browse units (BU), total canopy volume and total browseable volume (Smith and Hardy, 1999).

Quality of browses

Leaf samples of browse species were randomly taken for laboratory determinations, namely for the analyses of crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF). Cellulose, hemicellulose and lignin contents were derived from the ADF and NDF contents. The procedure outlined by the Association of Official Analytical Chemists (AOAC, 1995) was used for the chemical analyses.

Data analyses

Calculation of Tree Equivalence (TE)

A TE denotes a 1.5 m tree reachable by browsing goats. Tree equivalent for a sample site was calculated as a product of browse species density (in the sample area of 200 m^2) and height of species normalized by 1.5 m. TE was then expressed on hectare basis.

Calculation of browsing capacity

Browsing capacity of browse species was estimated from the quantity of browse available from the ground level to a height of 1.5 m. This is the zone reachable by browsing goats.

A mature West African forest type goat (25kg) was taken as the standard browse animal in this study; hence, browsing capacity was calculated based on the quantity of browse produced by browse species in the 1.5 m high browsing zone. Browsing capacity estimation was based on the evaluation of mean canopy radius, canopy volume, browse volume and browse unit (Smith and Hardy, 1999).

Mean canopy radius of each browse species was calculated as follows:

$$RAD = [\{(H_T - H_L)/2\} + R]/2 \quad (1)$$

where:
RAD = Mean canopy radius (m).
H_T = Plant height from ground level (m).
H_L = Lower canopy height, height from ground to lowest part of canopy (m).
R= Average horizontal radius of canopy.

The canopy volume (m^3) was calculated as follows assuming canopy to be spherical:

$$Vol = [(4 / 3) * (22 / 7) * (RAD^3)] \quad (2)$$

Browse volume (Vol_B) is the volume of the canopy of browse species below the 1.5 m reachable height for goats. It was calculated as:

$$Vol_B = [((22 / 7) * h^2) / 3] * [3 * RAD) - h] \quad (3)$$

where:
Vol_B = Browseable volume (m^3)
$h = (1.5 – H_L)$ (m)
H_L= Lower canopy height (m) from equation (1)

Equation (3) above was used to calculate browseable volume when the mid-canopy height was greater than the reachable browseable height of 1.5 m by goats. For browse species with mid-canopy height less than the reachable browse height of 1.5 m, equation (3) was used to first calculate the unbrowseable canopy volume above 1.5 m and

the browseable volume was determined by subtracting the unbrowseable volume from the total canopy volume for that browse species.

The BU was obtained by dividing the Vol_B by 0.94 m^3 (the mean canopy volume). Smith and Hardy (1999) found the total BU requirement per year for a mature goat of 50 kg in similar agro-ecological conditions is 1500. Since the weight of a mature forest type goat is half the weight used in their study, it was assumed that the BU requirement for the forest type goat per year will be 750. The area of browse resources required by one mature goat per year was obtained by dividing the minimum BU requirement by the mean BU supplied per hectare for the entire Coastal Savannah rangeland. Browse requirement was standardized to Tropical Livestock Units (TLU), assuming that, one TLU of 250 kg was equal to 10 mature forest type goats each having 0.1 TLU based on metabolic weight (LEAD, 2010). Thus, browse requirement for one mature goat was multiplied by ten (10) to express it per TLU.

Results

Browse capacity calculation

Browse unit

Using the mean canopy volume of 0.94 m^3 and equations (1), (2) and (3), it was observed that BU per ha ranged from 0 to 1,140.7 with a mean of 447.1. The overall mean BU per ha gave a browse capacity for one TLU as 16.8 ha per year or 1.4 ha per month in the dry season (Table 1).

Quality of indigenous browse species

The chemical analyses of the browse species (Table 2) indicated that CP content ranged from 85.4 (*Diospyros glomerata*) to 163.5 g/kg DM (*Securinega virosa*), NDF ranged from 244.0 (*Securinega virosa*) to 488.2 g/kg DM (*Dichrostachys glomerata*), ADF ranged from 121.0 (*Securinega virosa*) to 329.8 g/kg DM (*Dichrostachys glomerata*), cellulose content ranged from 77.9 (*Capparis erythrocarpos*) to 171.8 g/kg DM (*Mellittia thonningii*), hemicellulose ranged from 97.5 (*Zanthoxylum xanthoxyloides*) to 219.0 g/kg DM (*Mellittia thonningii*) and lignin content ranged from 39.1 (*Securinega virosa*) to 171.8 g/kg DM (*Dichrostachys glomerata*).

Discussion

Browse capacity of the Coastal Savannah rangeland

A very important requirement for livestock production is to balance stocking rate with grazing and browsing capacity (Smit, 2005). The browsing capacity of trees and shrubs depend on the palatability and quantity of browse materials they produce in the 1.5 m high browsing zone, since small stocks cannot browse beyond this height (Smith and Hardy, 1999). However, because of the difficulty in quantifying browse component of the range, many rangeland scientists do not take their contribution to forage production in the overall estimation of carrying capacity (Peyre de Fabregues, 1975). Carrying capacity is defined as the maximum possible stocking of herbivores that a rangeland can support on a sustainable basis (FAO, 1991). Hence, the estimation of carrying capacity of a range resource without the contribution of browses is likely to produce erroneous results. This study has however revealed that, the Coastal Savannah rangeland of Ghana produces about 447.1 BU per ha, translating into browsing capacity of 16.8 ha per year per TLU-1 or 1.4 ha per month per TLU-1. This is an indication that in the critical dry months of December to February when the quality of grazing materials have fallen to the lowest levels, ruminant livestock can survive on indigenous browse species for their supply of protein and energy.

Quality of indigenous browse species

Crude protein content

The CP contents obtained from chemical analyses of the leaves of the browse species ranged from 85.4 (*Diospyros abyssinica*) to 161.2 g/kg DM (*Urena lobata*). Voluntary intake of browses by ruminant livestock is influenced to a large extent by the dietary CP content (Devendra, 1991). The research findings of Dicko and Sikena (1991) indicated that CP content of most browse trees and shrubs exceed 100 g/kg DM even in the dry season. The results of this study were very

Table 1: Browse units and browsing capacity estimates

Site name	BU ha-^{1}	Site name	BU ha-^{1}
Sege Koni	1140.7	Agortor	785.9
Lalokpo	1071.5	Kpakyiridor	923.3
Ayisah	0	Dawa	8.2
Ada Luta	174.9	Bundase Wayo	278.8
Amu-yaw Kofe	376.6	Salom	421.6
Kpeyibor	229.3	Yomann site 2	401.1
Yomann site 1	0		
	Total	5811.9	
	Average	447.1	
	Stdev	404.5	

Browsing capacity is 16.8 ha year-^{1}TLU-^{1} or 1.4 ha month-^{1}TLU-^{1}

Table 2: Determinants of quality of the native browse species sampled in the dry season in the Coastal Savannah Plains of Ghana (g/kg DM).

Browse species	CP	NDF	ADF	Hemicellulose	Cellulose	Lignin
Securinega virosa	163.5	244.0	121.0	123.0	80.7	39.1
Dichrostachys glomerata	131.6	488.2	329.8	158.4	113.3	171.8
Diospyros abyssinica	85.4	467.4	264.3	203.1	130.1	112.4
Flacourtia flavescens	118.3	330.5	198.7	131.8	90.5	85.7
Zanthoxylum xanthoxyloides	90.4	296.0	198.5	97.5	117.7	61.9
Capparis erythrocarpos	127.6	386.6	187.8	198.8	77.9	107.2
Mellittia thornningii	92.9	473.1	254.1	219.0	174.9	79.2
Urena lobata	161.2	473.0	257.3	215.7	152.1	90.7
Mean	121.4	394.9	226.4	168.4	117.2	93.5
Stdev	30.4	95.0	63.0	47.0	34.0	39.0

close to their findings. Apart from the CP values for *Zanthoxylum xanthoxyloides* (90.4 g/kg DM), *Mellittia thonningii* (92.9 g/kg DM) and *Diospyros abyssinica* (85.4 g/kg DM), the CP contents for the rest of the species were higher than 100 g/kg DM (Table 2). Fianu *et al.* (1972) however observed slightly higher CP values of 150 to 200 g/kg DM in the dry season. Even though they worked on browse species found in the Coastal Savannah rangeland, their marginally higher CP values could be attributed to the fact that they investigated on two non-native leguminous browse species (Griffonia spp. and Baphia spp). According to Norton (1994a), forages containing less than 13.0 g/kg DM of N or 80.0 g/kg DM CP cannot provide the minimum ammonia levels required by ruminant livestock. With a CP range of 85.4 to 161.2 g/kg DM and a mean value of 121.4 g/kg DM, the indigenous browse species in this current study can contribute meaningfully to ruminant livestock feeding in the critical dry periods of the year. Apart from CP content, another measure of fodder quality is the plant cell wall constituents (Minson, 1990).

Cell wall constituents

Neutral Detergent Fibre is a measure of the cell wall constituents namely; cellulose, hemicellulose and lignin. In general, the NDF content of forages measure above 300.0 g/kg DM, and the higher the fiber content, the lower the energy content of the forage (Schroeder, 1996). Meissner *et al.*(1991) reported that the safe upper level of NDF to guarantee adequate intake of forage is 600 g/kg DM. Results of this

study gives the NDF range of the indigenous browse species from 244.0 (*Securinega virosa*) to 488.2 g/kg DM (*Dichrostachys glomerata*) with a mean value of 394.9 g/kg DM. This was slightly lower than that obtained by Sarkwa (2008) who reported a range of 278.0 (*Moringa exasperate*) to 598.0 g/kg DM (Baphia nitida). The mean NDF value of 394.9 g/kg DM obtained in this study was higher than that obtained by Addo-Kwafo (1996) in his work with Albizzia lebbek and lower than 505.0 g/kg DM obtained by Sottie *et al.* (1998). The generally low NDF values of all the indigenous browse species in the dry season (below the upper limit of 600.0 g/kg DM) is an indication that these species are good browse materials that can provide energy for ruminant livestock. Norton (1994b) reported that low NDF values for tree forages (200.0 to 350 g/kg DM) lead to high digestibility. Schroeder (1994) also indicated that as NDF values decline, DM intake by ruminant livestock increase.

Hemicellulose ranged from 123.0 (*Securinega virosa*) to 219.0 g/kg DM (*Mellittia thonningii*) with a mean value of 168.4 g/kg DM. Addo kwafo (1996) reported a mean hemicellulose content of *Albizzia lebbeck* to be 148.2 g/kg DM while Taiwo *et al.* (2009) working on seventeen different browse species reported a mean hemicellulose value of 322.4 g/kg DM. Hemicellulose content of this study falls between those reported by these two authors. Cellulose content obtained ranged from 77.9 (Securinega virosa) to 174.9 g/kg DM (Mellittia thonningii) with a mean value of 117.2 g/kg DM. This result is lower than that obtained by Sottie *et al* (1998) and Addo-Kwafo (1996) who obtained 196.0 g/kg DM and 184.7 g/kg DM mean cellulose contents respectively. Lignin content obtained ranged from 39.1 (*Securinega virosa*) to 171.8 g/kg DM (*Dichrostachys glomerata*) with a mean value of 93.5 g/kg DM. This result was very close to the 107.0 g/kg DM mean lignin content obtained by Sottie *et al.* (1998) but lower than the 124.4 g/kg DM obtained by Ikhimioya *et al.* (2007). The ADF content obtained in this study ranged from 121.0 (*Securinega virosa*) to 329.8 g/kg DM (*Dichrostachys glomerata*) with a mean value of 226.4 g/kg DM. This mean ADF content obtained in this study was lower than those obtained by several authors (Addo-Kwafo, 1996; Sottie *et al.,* 1998; Ikhimioya *et al.,* 2007).

The differences in cell wall constituent values obtained in this study can be attributed to differences in browse species used and differences due to variations in sites of harvest within the savannah biome.

Shrubs and small tree browses are particularly valuable as feed sources during the dry season. Browse constitutes at least 30 % of a domestic ruminants' diet in the dry season and about 5 % in the rainy season (Le Houérou, 1987). According to Rose Innes and Mabey (1964), thicket species on the Accra plains have high acceptability and intake rates. However, apart from *Securinega virosa* and *Mellittia thonningii* among these species that have received some research attention in Ghana, much has not been done with the rest of the browse species.

Conclusion

This research has revealed that, the Coastal Savannah rangeland of Ghana is endowed with indigenous browses that maintain a high nutritive value in the dry season and have a huge potential in curbing the dry season feed deficit. With an estimate of browsing capacity, we have a holistic understanding of forage supply in the Coastal Savannah rangeland that will lead to the introduction of more functional interventions to boost the livestock industry.

Impact

Tropical Coastal Savannahs are endowed with woody plant species that contribute to livestock feed particularly in the dry periods when rainfall is inadequate or completely absent. These woody species have not had adequate attention by researchers. This study therefore identifies the indigenous browse species in the Coastal Savannah rangelands of Ghana, evaluates their nutritive value in the dry season when the quality of grasses have reduced tremendously and estimates how many ruminant livestock can depend on these browse species for feed without causing any deterioration to the

natural ecosystem.

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EFFECT OF VARYING CRUDE PROTEIN LEVELS ON THE PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILER CHICKEN IN THE HUMID TROPICS

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Summary

This study was conducted to determine the effect of varying crude protein (CP) levels on the performance and carcass characteristics of broilers. In a 56 days feeding trial, 252 day old chicks were allotted to four dietary treatments. Each treatment consisted of 3 replicates of 21 birds each. Birds on each treatment (Trt) were fed varying CP (%) in a 3 stage feeding plan consisting of starter (0-3weeks), grower (3-6weeks) and finisher (6-8 weeks) and CP was reduced step wisely by 2% except in Trt IV as follow: Trt I, 25, 23 and 21; Trt II, 23, 21 and 19; Trt III 21, 19 and 17; Trt IV, 19 throughout the 3 stages and diets were based mainly on maize and soya bean. Daily weight gain and feed conversion were largely unaffected by the imposed dietary CP treatment during the starting, growing and finishing stages, but feed intake was significantly affected during the multi-stage feeding plan. Birds on Trt III, IV and IV had significantly ($P<0.001$) higher feed intakes during starter, grower and finisher phases respectively. However, the daily gain of birds on Trt III (21-19-17) was significantly depressed, when fed 17% CP at the finishing stage. Varying CP levels had no significant ($P > 0.05$) effect on dressing, breast and drumstick percentages determined (as % of live weight) at week 8. It was concluded that 19% CP may be sufficient for raising broilers with 36g daily gain from start to finish on corn-soya bean diet.

Keywords: Broiler, carcass portions, nutrient requirement, multi-stage feeding, breast yield, protein.

EFFET DE LA VARIATION DE TAUX DE PROTÉINES BRUTES SUR LA PERFORMANCE ET LES CARACTÉRISTIQUES DES CARCASSES DES POULETS DE CHAIR DANS LES TROPIQUES HUMIDES

Résumé

Cette étude a été effectuée dans le but de déterminer l'effet de la variation de taux de protéines brutes (CP) sur la performance et les caractéristiques des carcasses de poulets de chair. Dans un essai alimentaire de 56 jours, 252 poussins d'un jour ont été assignés à quatre traitements alimentaires. Chaque traitement consistait en 3 répétitions de 21 oiseaux chacun. Dans chaque traitement T les oiseaux ont été nourris avec des CP (%) variéssuivant un régimealimentaire en 3 étapes, constitué d'un régime de démarrage (0 à 3 semaines), de croissance (3-6 semaines) et de finition (6-8 semaines), et la CP a été raisonnablement réduite par étape de 2%, sauf dans le T4,de la manière suivante : T1, 25%, 23% et 21%; T 2, 23%, 21% et 19%; T321%, 19% et 17%; T4, 19% tout au long des 3 étapes, et les éléments de base des régimes alimentaires étaient principalement le maïs et le soja.Le gain pondéral quotidien et la conversion alimentaire ont été,dans une large mesure, non affectés par le traitement pendant les phases de démarrage, de croissance et de finition, mais l'ingestion d'aliments a été significativement affectée au cours du plan alimentaire multi-étapes. Les oiseaux soumis aux T3 et T4, avaient des consommations alimentaires significativement ($P <0,001$) plus élevées durant les phases de démarrage, de croissance et de finition. Cependant, le gain pondéral quotidien des oiseaux sur T3 (21-19-17) était significativement réduit, lorsque leur nourriture comportait une CP à 17% au stade de la finition. Différents taux de CP n'ont pas eu d'effet significatif ($P> 0,05$) sur les pourcentages du rendement carcasse, rendement filet et rendement pilon déterminés (en% du poids vif) à la semaine 8. Il a été conclu que la CP de 19% peut être suffisante pour élever les poulets de chair avec un gain quotidien de 36g de bout en bout sur un régime de maïs-soja.

Mots-clés: Poulet de chair, Portions de carcasse, Besoin nutritionnel, Alimentation en étapes, Rendement filet, Protéine.

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Introduction

The National Research Council (NRC; 1994) guide on nutrient requirement for poultry cannot apply to all chicken under all rearing conditions and climatic conditions. At present Nigeria has no unified nutrient requirement publication for any of her farm animals and this has led to reliance on the NRC requirement specifications for ration formulation for monogastric animals. The need for regular re-evaluation of requirement of broilers also stems from the fact that new genotypes/strains of birds are being developed and improvement in existing processing feed techniques and development of improved crop cultivars/varieties often lead to higher nutrient density, higher nutrient recovery from feed and better bioavailability of nutrients in feed ingredients. Studies on requirements therefore need to be re-examined on regular basis due to these changing genetic and production scenarios. Some earlier reports have indicated that the requirement for crude protein for broiler in the tropics may be higher than that of the temperate region (Olomu and Offiong, 1980; Olomu, 1995; Onwudike, 1983), but these estimations were more than 15 years old. It is also a well known fact that, poultry species (broiler and layers) do not attain the high growth rate and laying performance often recorded in the temperate region when raised in the tropical environment even though the genotypes may be the same. Growth consists mainly of water, protein, fat and mineral accretions. If the growth rate is lower in broilers raised under the humid tropics, then it is questionable if the requirements are the same if the levels of intended or realised performance are at variance. The typical body weight changes of NRC (1994) for broiler of 1900g at 6 weeks or 2800g at 8 weeks for unsexed broiler is hardly attained in Nigeria even with supplemental lightening to prolong day length and allow for more feed intake. Similarly, constant review of nutrient requirements of food producing animals is to increase precision in feeding and at the same time reduce the environmental impact of animal production by decreasing the output of nutrients in faecal and urinary excretion in manure especially those of phosphorus and nitrogen which are the major culprits in the contamination of surface and ground water (Jongbloed and Lenis, 1998; Hudson *et al.* 2000; Kerr, 2003). It is therefore the objective of this study to determine the effect of varying levels of crude protein on the performance and carcass characteristics of broiler chicken.

Materials and Methods

252 day old Marshall broiler chicks obtained from Obasanjo hatchery, Ibadan were randomly allotted to four dietary treatments. Birds were housed on floor pens and managed in a manner similar to those previously described (Fatufe and Matanmi, 2008; Matanmi and Fatufe, 2008). Each treatment (Trt) consisted of 3 replicates of 21 birds each. Birds on each treatment were fed crude protein (CP) in a 3 stage feeding plan consisting of starter (0-3weeks), grower (3-6weeks) and finisher (6-8 weeks) stages and CP was reduced step wisely by 2% from starter to finisher except in treatment 4 as follow: Trt I, 25, 23 and 21; Trt II, 23, 21 and 19; Trt III 21, 19 and 17; Trt IV, 19 throughout the 3 stages. The range of crude protein used in the present study was within the range of those examined in previous studies 3, 4 in the study of crude protein requirement of broilers in the humid tropics and crude protein was step wisely reduced by 2% in the grower and finisher phases, because the protein requirement of broilers is known to decrease with age, partly because of increase in feed intake, which tend to compensate for reduced protein levels in diets and the physiological development of the gastro-intestinal tract, which also results in improved nutrient digestibility with increasing age (NRC, 1994; Gesellschaft für Ernährungsphysiologie (GfE), 1999). Soya bean meal was the sole protein source, while cassava flour (human grade cassava meal, mainly starch detoxified to non- detectable levels of hydro-cyanide content through fermentation, sieved and later fry-dried) was used to replace soya bean meal in order to achieve lower level of crude protein (table 1). Consequently, as the level of soya bean meal (protein source) was exchanged for non-protein filler, the level of crude protein

decreased accordingly to the intended levels. Cassava flour was chosen to modulate protein levels due to its near zero content of protein and amino acid contributions and also to ensure that soya bean meal remain the major source of nitrogen in all diets. All diets were formulated to contain approximately the same amount of energy of 3000 kcal/kg metabolisable energy. The calculated compositions of the test diets are shown in table 2. Amino acid contents of formulated feed were calculated based on the published values in Aminodat 1.1, Degussa AG, Germany. Birds were fed the appropriate diet depending on their growth phase and the intended protein supply. Feed and water were offered ad libitum. Birds were weighed individually on weekly basis using a precision scale and the experiment lasted for 8 weeks. At eight weeks, 3 individuals from each treatment having comparable body size to their treatment means were selected, slaughtered, dressed, cut into standard parts (eg. breast and drumstick) and portions were weighed separately. The proximate composition of the feed ingredients used for feed formulation was determined using the AOAC (1990) procedure. Data were subjected to one way analysis of variance and mean separation for significance was carried out using Duncan multiple range tests with SPSS 13.0 version for windows. Levene's test for homocedasticity was carried out using the same software.

Results

The analysed proximate compositions of the feed ingredients used for feed formulation are in good agreement with the published values and differences are not beyond that of analytical error. However, diet formulations (table 2) were based on the analysed proximate constituent values of feed ingredients. As the level of soya bean meal (protein source) was exchanged for non-protein filler, the level of crude protein decreased accordingly to the intended level. The assumption of homogeneity of variance was tested using Levene's test and results indicated a non-significant difference ($p > 0.05$) for the performance parameters (daily gain, feed intake, feed conversion and carcass portions) measured in the present study. Growing chicken did not significantly respond to varying levels of dietary crude protein for most performance characteristics. The result of performance characteristics of broilers fed varying levels of dietary crude protein is presented in table 3. Daily weight gain and feed conversion ratio were largely unaffected by the imposed dietary treatment during the starting, growing and finishing stages, but feed intake was significantly affected during the multi-stage feeding plan. Birds on Trt III, Trt IV and Trt IV had significantly ($P<0.001$) higher feed intakes during starter, grower and finisher phases respectively. However, the daily gain of birds on Trt III (21-19-17) was significantly depressed, when fed 17% CP at the finishing stage. There was no significant difference in the dressing percentage and relative carcass composition (portion as percentage of live weight) between birds placed on varying crude protein levels at the end of the study at 8 weeks. The thigh and drumstick and the breast portions were on the average 22.5 and 16.8% respectively.

Discussion

Growing chicken did not significantly respond to varying levels of dietary crude protein for most performance characteristics. Daily weight gain and feed conversion ratio were largely unaffected by the imposed dietary treatment during the starting, growing and finishing stages, but feed intake was significantly affected during the multi-stage feeding plan. Surprising, the daily gain of birds on Trt III (21-19-17) was significantly depressed, when fed 17% CP at the finishing stage. Similarly, birds fed 19-19-19% CP had significantly higher feed intake than the usual 23-21-19% CP (NRC, 1994), however, increase in feed intake also resulted in higher growth rate, therefore FCR was not compromised. The depressed growth rate on Trt III and the higher feed intake on Trt IV could possibly be due to the reduced protein content of the diet. It is well known that reduced crude protein intake may increase feed intake in birds (Smith and Pesti, 1998; Sklan and Plavnik, 2002), but the depressed feed intake observed on Trt III (21-19-17) at the finishing phase when 17% CP was fed may imply that this crude protein level is too

Table 1: Gross composition of experimental diet (g/kg)[1]

Feeding plan[2]					
Starter	TrtI	Trt II	Trt III	Trt IV	
Grower		Trt I	Trt II	Trt III; Trt IV	
Finisher			Trt I	Trt II; Trt IV	Trt III
Ingredients	**25%CP**	**23%CP**	**21%CP**	**19%CP**	**17%CP**
Maize	508.1	508.1	508.1	508.1	508.1
Soybean meal	450	402	356	312	270
Cassava flour	0	48	94	138	180
Others3	41.9	41.9	41.9	41.9	41.9

[1] Trt I, II, III and IV represent Treatments I, II, III and IV respectively
2Birds were fed CP (%) diets as follow: Treatment 1: 25, 23 and 21; Treatment 2: 23, 21 and 19; Treatment 3: 21, 19 and 17; and Treatment 4: 19, 19 and 19 (same diet throughout the feeding trial).
3Same for the five diet (g/kg) bone meal 35, Salt 2.5, DL-methionine 1.4 and premix 3.

Table 2: Analysed composition of broiler chicken diet (g/kg)

Parameter[1]	**25%CP**	**23%CP**	**21%CP**	**19%CP**	**17%CP**	**Av. Ratio to Lys**
ME kcal/kg	2960	2984	3007	3029	3050	
Crude protein	246	226	206	188	170	
Crude fibre	38.9	39.5	39.6	40.6	41.1	
Lysine	13.5	12.2	11.0	9.8	8.6	100
Threonine	9.7	8.8	8.0	7.2	6.5	73
Methionine	5.2	4.9	4.6	4.3	4.1	43
Tryptophan	2.9	2.6	2.4	2.1	1.9	22
Isoleucine	11.5	10.4	9.4	8.5	7.5	86
Valine	12.2	11.1	10.1	9.1	8.2	92
Leucine	21.1	19.4	17.8	16.3	14.8	163
Phenylalanine	12.5	11.4	10.3	9.3	8.4	94
Histidine	7.0	6.4	5.8	5.2	4.7	53
Arginine	17.8	16.1	14.5	12.9	11.4	132

[1]Birds were fed CP (%) diets as follow: Treatment 1: 25, 23 and 21; Treatment 2: 23, 21 and 19; Treatment 3: 21, 19 and 17; and Treatment 4: 19, 19 and 19 (same diet throughout the feeding trial).

low for the birds. About 4% CP was reduced during the starting and 2% CP at growing stages respectively, from the 19-19-19% CP compared to the 23-21-19 % CP (similar to NRC recommendation for broiler). Protein and energy sources constitute more than 85% cost of production cost of broiler feed. Any reduction in protein content in feed without any adverse effect on performance will imply additional saving on protein cost for broiler production in the humid tropics. However, chicken were more at home on 19-19-19CP diet and gained more weight than all other diet combinations. The environmental benefit of low nitrogen excretion through dietary protein manipulation may also speak for low protein diets, especially in the hot humid tropics, where heat increment associated with the excretion of excess nitrogen may further exacerbate the already not too comfortable rearing temperature which is usually higher than the ideal 22-25°C observed in the temperate region.

It has been observed from previous studies that increasing the amino acid density (by feeding higher crude protein levels) in broiler diet may lead to improved breast yield, lower feed conversion and less feed consumption in modern genetic strains with high genetic potential for lean deposition.

Increasing the crude protein from 20.5 to 26% in male broilers growing from 14 to 35 days old clearly resulted in improved weight gain, feed conversion, breast meat yield, and reduced abdominal fat content (Vieira *et al.,* 2004). Other studies in which crude protein (and amino acid concentrations) was increased beyond the traditional levels were also in agreement with above study (Eits *et al.,* 2003; Corzo *et al.,* 2005). Reducing crude protein and amino acid density in broiler's diet have also been reported to reduce meat and breast yields without an effect on weight gain (Corzo *et al.,* 2005). Conversely, feeding above 19% crude protein (19-19-19 %CP) from start to finish from the present study did not result in improved growth performance in terms of feed conversion, breast meat yield and weight gain. It is probable that the broiler strain used for the actual study has less need for high protein diet because of its maximum daily gain, which was quite below the NRC (1994) average (36 vs. 50 g/day). Our result is in agreement with results from other studies, in which the positive effect of feeding high crude protein or lysine equivalent in the diet on growth performance was not detected (Kidd *et al.,* 2005; Dozier *et al.,* 2006).

Onwudike (1983) conducted a factorial experiment with Ross broilers age 3 weeks to 12 weeks with 4 levels of crude protein (%; 20, 22, 24 and 26) and 3 levels of metabolisable energy (kcal/kg; 2800, 3000 and 3200) and concluded that the 22% crude protein and 3000 kcal/kg was adequate for broilers raised under the tropical environment. Recalculation the 22% crude protein and 3000 kcal/kg diet made up of maize (57%), groundnut cake meal (23%), fishmeal (12%) and rice bran (3.95%) and non-protein ingredients (3.55%) of Onwudike (1983) showed a marked resemblance to 19% crude protein used in the present study in terms of essential amino acid composition (Table 2). The total amino acid composition (%) compared to the present study were lysine (1.09 vs. 0.98), methionine (0.44 vs. 0.43), methionine plus cysteine (0.77 vs. 0.74), threonine (0.78 vs. 0.72), tryptophan (0.21 vs. 0.21) among others. Similarly recalculation of the 22% CP diet of Onwudike (1983) for digestible amino acid content also bear a resemblance with the 19% CP. The digestible amino acid contents (%) of this diet compared to the present 19% diets were lysine (0.92 vs. 0.88), methionine (0.39 vs. 0.41), threonine (0.68 vs. 0.64), arginine (1.65 vs. 1.17), isoleucine (0.73 vs. 0.73), leucine (1.67 vs. 1.53) and valine (0.91 vs. 0.83). The daily gain of broilers on 19% CP in the present study of 36g/day was slightly higher than 34 g/day of broilers on 22% CP reported by Onwudike

Table 3: Performance of broiler chicken fed varying levels of crude protein (0-8weeks; n = 3)

Parameters[1,2]	Trt I	Trt II	Trt III	Trt IV	SEM	P (ANOVA)
0-8 weeks						
Initial body weight (g/bird)	40.6	40.5	40.5	40.5	0.01	-
Final body weight (g/bird)	1950^{ab}	1962^{ab}	1903^{b}	2059^{a}	19.8	0.041
Body weight gain (g/bird/day)	34.1^{ab}	34.3^{ab}	33.3 ^{b}	36.0^{a}	0.35	0.041
Feed intake (g/bird/day)	82.2^{a}	77.8^{b}	82.3^{a}	82.0^{a}	0.31	<0.001
Feed conversion ratio	2.46	2.29	2.50	2.45	0.05	0.474
Others[3]						
Feed intake (g/bird/day; Starter)	37.7^{b}	37.2^{c}	38.2^{a}	37.3^{c}	0.08	<0.001
Feed intake (g/bird/day; Grower)	96.3^{bc}	97.8^{b}	95.7^{c}	100.0^{a}	0.34	<0.001
Feed intake (g/bird/day; Finisher)	125^{ab}	105c	123b	128a	0.95	<0.001
Body weight gain (g/bird/day; Finisher)	41.0 ^{ab}	40.4 ^{ab}	37.0^{b}	45.0^{a}	1.25	0.040

[1] Trt I, II, III and IV represent Treatments I, II, III and IV respectively

[2]Birds were fed CP (%) diets as follow: Treatment 1: 25, 23 and 21; Treatment 2: 23, 21 and 19; Treatment 3: 21, 19 and 17; and Treatment 4: 19, 19 and 19 during starting (0-3 weeks), growing (3-6 weeks) and finishing (6-8 weeks) respectively.

[3]Daily weight gain, feed conversion ratio and feed intake were analysed within each phase of start, grow and finish; only significant data are shown

Table 4: Effect of varying crude protein on the portions of broilers at 8 weeks of age (% of live weight)

Parameters[1,2]	Trt I	Trt II	Trt III	Trt IV	Pooled SEM	P(ANOVA)
Dressing percentage	71.39	69.98	69.02	68.9	0.698	0.628
Breast	16.96	16.74	16.14	17.42	0.369	0.732
Thigh and drumstick	21.63	21.53	21.54	22.98	0.301	0.263
Back	12.04	13.79	13.02	13.48	0.347	0.340
Wings	9.33	9.26	8.65	9.42	0.261	0.775
Liver	2.04	1.87	2.33	2.40	0.153	0.634
Gizzard	2.14	2.05	2.15	2.81	0.122	0.073
Head and neck	8.01	8.54	6.78	8.87	0.48	0.490
Shanks	5.59	4.96	5.18	5.54	0.140	0.368

[1]Trt I, II, III and IV represent Treatments I, II, III and IV respectively
[2]Birds were fed CP (%) diets as follow: Treatment 1: 25, 23 and 21; Treatment 2: 23, 21 and 19; Treatment 3: 21, 19 and 17; and Treatment 4: 19, 19 and 19 during starting (0-3 weeks), growing (3-6 weeks) and finishing (6-8 weeks) respectively.

(1983). The differences in the published values of crude protein requirement of broilers in the tropics (Onwudike, 1983; Olomu, 1995, Oyedeji et al., 2005) may be partly explained by the total and digestible amino acid contents. The 36g/day daily gain of broilers on 19% CP in the present study is also considerably low to NRC (1994) average of broiler from start to finish of 50g/day, but feeding higher level of dietary protein and by extension higher level of amino acids from intact protein sources with highly digestible corn-soya bean meal in this study did not result in higher daily gain.

The major relative carcass portion was not influenced by the variation in crude protein concentration. Breast and, thigh and drumstick contributed 17.42 and 22.98% to the live weight in Marshall Broiler respectively (table 4). This was quite similar to what was observed in Anak broilers in a previous study (Egbunike *et al.,* 2009) in which thigh and drumstick and the breast portions constituted 20.9 and 18.5% of the live weight respectively, in broilers fed optimal crude protein concentration. Even though the proportion of these two portions appear to be slightly different for the two strains of broiler chicken, the overall contribution of these two very important chicken cut portions to the live weight were very close (39.39% (Anak) vs. 38.74 (Marshall)).

Conclusion

It was concluded that the crude protein requirement of broilers reared in the humid tropics with daily gain of 36g/day may not exceed 19% CP from start to finish stages and assuming 85% digestibility for the crude protein of maize-soya bean meal based diet, the digestible protein of broilers with this growth rate may not be more than 16%. Similarly, lowering crude protein to 19% CP from start to finish phases in the current study has no adverse effect on carcass yield and economically important carcass portions such as breast and thigh and drumstick.

Impact

Broiler chickens in the humid tropics are mainly fed based on the feed specification of National Research Council (NRC), USA, but the environment in which these specifications were derived is quite different from that of the tropics. Also, broilers reared in the tropics do not attain the high growth rate as those in the temperate regions, which makes feeding then the same level of nutrient questionable. This study suggests that the crude protein requirement of broilers raised in the humid tropics may be lower than the NRC. Precise feeding of broilers will reduce the cost of feeding broiler and also minimise nitrogen and phosphorus excretions into the environment.

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A SURVEY FOR ANTIBODIES AGAINST CURRENT INFECTION OF FOOT-AND-MOUTH DISEASE VIRUS IN SUDANESE CATTLE, SHEEP AND GOATS USING NEUTRALIZATION TEST

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Summary

A screening format of serum neutralization (SN) test was used to screen Sudanese cattle, sheep and goats sera against current infection of type "O" and "SAT2" foot-and-mouth disease (FMD) viruses. The format was easy to perform; reading of results was objective and it detected high seropositivity in distinct test groups of cattle; as high as 82.6% and 43.7% for types "O" and "SAT2" respectively. Similar figures to these have been reported using the sensitive liquid-phase blocking ELISA (LPBE) in recent different occasions.

Results obtained by the screening format confirmed recent serological findings obtained by the LPBE. Serotype specific antibodies against types "O" and "SAT2" were significantly higher in tested cattle (63.15% and 20.63% respectively) than in tested sheep (9.16% and 1.16% respectively). None of the examined goats proved to be positive to either serotypes (n= 35 and 27 respectively). Seroprevalence of antibodies to the long known predominant type "O", unlike seroprevalence of type "SAT2" antibodies, was markedly lower in local (33.3%) than in cross (63.15%) tested cattle breeds. In Western Sudan, where local breeds of cattle prevail, seroprevalence of antibody to the moderately prevalent type "SAT2" (40%) even surpassed that of antibody to type "O" (25%).

It could be concluded that unapparent FMD infection in sheep and goats in Sudan occurs secondary to infection in cattle and no separate cycle of FMD infection occurs in these species. The results were highly suggestive of a diminished role of sheep and goats in the epidemiology of FMD within the epidemiological setup of Sudan. Likewise, results were suggestive of development of natural resistance in local cattle breeds to the earliest country reported type "O" infection.

Key words: FMD- Sudan- serum neutralization- cattle, sheep and goats

ETUDE DES ANTICORPS DU VIRUS DE L'INFECTION ACTUELLE DE FIÈVRE APHTEUSE CHEZ LES BOVINS, OVINS ET CAPRINS SOUDANAIS UTILISANT LE TEST DE NEUTRALISATION

Resume

Un test de séro-neutralisation (SN) a été utilisé pour détecter les infections de fièvre aphteuse des types O et SAT2 chez les bovins, ovins et caprins au Soudan.

Les résultats obtenus ont révélé de grandes différences de séroprévalence entre les bovins et les petits ruminants. Le type prédominant O connu depuis longtemps a montré une séroprévalence élevée (63,15%) chez les bovins par rapport à une séroprévalence de 9,16% et une séroprévalence nulle respectivement chez les ovins et les caprins. Le type SAT2 a montré une séroprévalence de 20,63% chez les bovins, et 0.01% chez les petits ruminants. En accord avec les résultats récents, il a été conclu que l'infection inapparente de fièvre aphteuse chez les ovins et caprins au Soudan survient secondairement à l'infection des bovins et qu'aucun cycle distinct d'infections de fièvre aphteuse ne se produit chez ces espèces. Les résultats ont été très évocateurs d'une diminution du rôle des ovins et caprins dans l'épidémiologie de la fièvre aphteuse dans la configuration épidémiologique du Soudan.

Les résultats ont également confirmé une résistance naturelle observée précédemment chez les races bovines locales à l'infection de type O signalée en premier dans le pays, outre les autres sérotypes circulants de la fièvre aphteuse La séroprévalence du type O détecté est nettement plus faible chez les races bovines locales (33,3%) par rapport aux races bovines croisées (63,15%) examinées, et la séroprévalence du type SAT2 chez les bovins du Soudan occidental (40%), où prédominent les races locales, était supérieure à celle du type O (25%) connu depuis longtemps.

Mots-clés : Fièvre Aphteuse- Soudan- séroneutralisation- bovins, ovins et caprins

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Introduction

Of animal viral diseases, foot-and-mouth disease (FMD) is the one with the most complex epidemiology. Multiplicity of virus types and the wide range of animal species contract natural infection are factors that play important role in this complexity. Furthermore, in enzootic areas of Africa, mild and subclinical FMD is not uncommon (Kitching, 2002). Sero-surveillances are of particular usefulness for determining the extent of these forms of mild infections in different species. The progressive control pathway (PCP) for FMD developed by the Food and Agriculture Organization (FAO) stipulates extensive sero-surveillances as a key activity to gain clear insight into the epidemiology of FMD and to develop a risk based control policy (FAO, 2011). Many ELISAs for structural proteins (SP) and for non structural proteins (NSP) serology, in addition to the serum neutralization (SN) test, has been developed and are widely used in these surveillances. In comparison to the SN test, ELISAs are easy to perform and considered by many workers as more reproducible. On the other hand, the SN test remains the golden standard for FMD serology (OIE, 2008).

In Sudan, clinical FMD was reported in cattle only while sheep and goats were known to undergo unapparent infection (Abu Elzein, 1983; Abu Elzein et al, 1987). Recent serological findings indicated the maintained activity of 3 serotypes; "A", "O" and "SAT2", though current infections of FMD in the years 2005, 2007, 2008 and 2010 were of the serotypes "O" and "SAT2" (Raouf *et al,* 2009; Habiela *et al,* 2010; Raouf *et al,* 2010; Anon, 2010). It indicated wide differences between sero-prevalence's in cattle and small ruminants. Serotype specific antibodies are much more prevalent in sera of cattle than in sera of small ruminants and follow more or less the same order in all species; "A", "O" and then "SAT2" (Habiela *et al,* 2010; Raouf *et al,* 2011). Also, among the significant recent findings was the lower sero-prevalence of type "O" antibodies in local cattle breeds, apart from other serotypes, in comparison to exotic and cross breeds; indicative of development of a natural resistance to the long predominant type "O" infection (Raouf *et al,* 2011). In these surveys the liquid-phase blocking ELISA (Hamblin, *et al* 1986) (LPBE) was used exclusively. On one hand, more surveys are required to extend these findings in the wide geographical area of Sudan while, on the other hand, the use of SN test, the golden standard, is noteworthy advantageous for support of such findings.

In the presented work, antibodies against currently circulating FMD viruses, "O" and "SAT2", were screened in different species of domestic ruminants using the SN technique. The work describes a screening format of SN test which is relatively easier to perform than the standard SN test. The format renders the technique of neutralization more suitable for surveillance of field sera and expected to ease the constraint on financial resources necessary for extensive surveillances by trimming down cost of the supply of screening ELISA kits. The result obtained by the screening format, so far, confirmed and supported the recent serological findings.

Serum samples:

A total of 250 sera were used in the study: 95 from cattle, 120 from sheep and 35 from goat. Of them, 180 sera; (49 cattle, 96 sheep and 35 goats) were collected from known animal breeds and known geographical locations in Western, Central and Eastern Sudan. The remainder, 70 sera; 46 cattle and 24 sheep, were collected from animals kept at a quarantine of a slaughterhouse in Khartoum state. All sera were collected in the year 2008. The source and breeds of animals at the slaughterhouse was unknown.

All animals had no vaccination history against FMD. Cattle were above one year old and sheep and goats were above 6 months old. Sera were collected in plain vaccutainers, separated by centrifugation, inactivated at 56°C for 30 minutes and kept at -20°C till use.

Reference sera:

Reference antisera against FMD virus types "O" and "SAT2", and reference negative bovine serum were obtained from the World Reference Laboratory (WRL) for FMD (Pirbright, UK). They were inactivated in similar manner to test sera.

Viruses:

Recent Sudanese FMD virus isolates of the serotype "O" and "SAT2", referred to as O-Jaz 1/08 and SAT2-Kh 2/08 (Raouf et al, 2010) were used throughout this work. These viruses were adapted to grow in cultures of calf kidney cell (CKC) through 11 and 12 passages respectively. The character of adaptation was defined, as the virus growth that effect absolute determination of titre of infective material. A further, one passage (O-Jaz 1/08) and four passages (SAT2-Kh 2/08) in BHK 21 cells were undergone before using in the SN procedure.

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. Stock virus titres were found to be stable under these conditions and varied little over a period of 1-3 months. Virus titration was done 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) and 0.0487% Na HCO_3 (W/V)}. Growth media for BHK 21 cells contained in addition 10% tris-buffer (0.05M) and 10% newborn calf serum (NBCS) (Sigma). Titres were calculated according to the method of Kärber (1931).

Serum Neutralization test:

The standard procedure of the quantitative microtest for titration of FMD antibody in sera as described in the OIE manual (2008) was applied using BHK 21 cells and 100 TCID50 of infective virus. Three columns were used for each serum and each serum dilution was tested in two wells while the third well was left as serum control. Sera were tested starting from 1/8 (final dilution of 1/16) to 1/1024 dilutions (final dilution of 1/2048). Titres were calculated according to the method of Kärber (1931). Titres equal to or above 101.5 were considered positive.

Screening format of the SN test:

In the format, sera were tested at final dilutions of 1/32 (10-1.5) and 1/64 (10-1.8) only. 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, in columns 11 and 12 of a micotitre plate.

Serum diluent (complete GMEM containing 10% tris-buffer) was distributed all through a u-bottomed microtitre plate; 95 µl to each well in rows A, C, E and G and 50 µl to each well in rows B, D, F and H. Six µl of a tested serum was added to each well of one pair in rows A, C, E and G. Each row would test 6 sera at dilution 1/16 (final dilution of 1/32). After mixing, 50 µl of the mixture was transferred from row A to B, from C to D, from E to F and from G to H using 12-channel microtitre pipettes and different tips for each 2 rows. The mixture was pipetted thourghly and 50 µl was discarded leaving 50 µl of dilution 1/32 (final dilution of 1/64). Fifty µl of a previously titrated virus stock, containing 100 TCID50, was added to each well except the 4 wells of the cell control which received in place virus diluent (complete GMEM). The virus control received no serum but serum diluent. The positive and negative serum controls were reference sera or local bovine sera of known positivity and with titre between 101.95 and 102.1. The plate was sealed with adhesive tape, lightly tapped and left at room temperature for one hour. At the end of which, 50 µl of suspension of BHK 21 cells, in the above described growth medium, was added. The cell suspension was sufficient to produce confluent monolayer 24 hours later. The plate was sealed with adhesive tape, incubated at 37 °C with a source of humidity and read microscopically 48 hours later. On the 3rd day post-seeding, it was stained with 0.1% crystal violet stain in 10% 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.

Results

Screening format:

Figures (1, 2 and 3) show titration and screening of sera. In titration assay

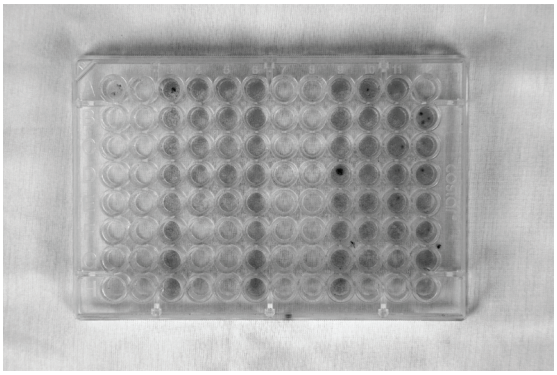

Figure 1: Quantitative microtest for titration of FMD antibody in serum.
Each serum was tested in 3 columns. Columns 3, 6, 9 and 12 were serum controls. Sera No. 1 and 3 were negative. Sera No. 2 and 4 were showing titres of 2.1 and 2.7 log 10 respectively.

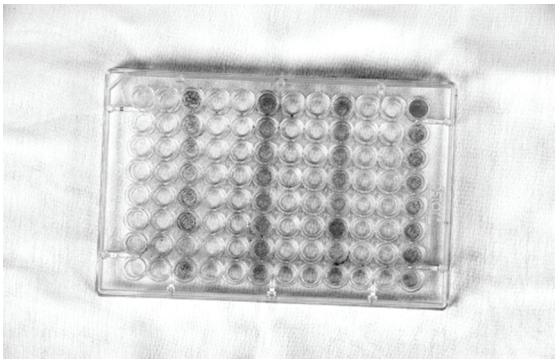

Figure 2: Quantitative microtest showing titration of negative sera.
Observe normality of serum controls in every case (columns 3, 6, 9 and 12)

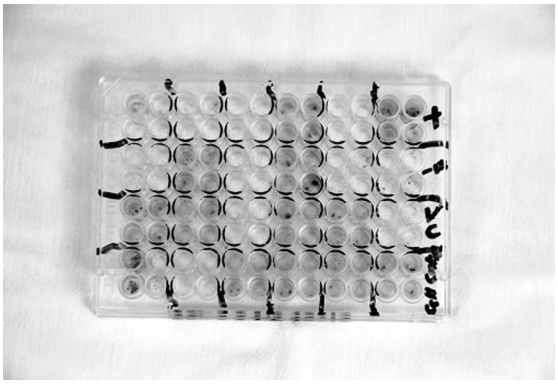

Figure 3: Screening format of VNT.
Each serum was tested in 4 wells (2 wells for final dilution of 1/32 and 2 wells for final dilution of 1/64). Column 11 and 12 were plate controls. Positive wells were stained and negative wells were empty.

(Fig. 1 and 2) the third columns (3, 6, 9 etc) represented tested serum controls. None of the sera titrated that included cattle, sheep and goats sera (n=25) showed any toxic effect on monolayer sheet of the BHK 21 cells, what eliminated the need for tested serum control in the screening format.

In the screening assay (Fig. 3), it is evident that discrimination of sera as positive or negative was highly objective. Discrepancy between microscopic and dye interpretation of results was 4% and all were detected in type "O" survey. Results were highly reproducible. In 12 rounds of the screening format, results of cell, virus, positive and negative serum controls were reproducible. About half of "SAT2" screened positive sera (n=6) and a number of type "O" negative and positive sera were repeatedly tested and produced the exact same results.

Table (1) shows the highest positivity detected for types "O" and "SAT2" in some test groups by the screening format.

Serum survey:

Results presented in table (2) show the difference between cattle and small ruminants in prevalence's of type "O" and "SAT2" antibody. Only one animal, out of 86 sheep and goats examined, was positive for the medium prevalent type "SAT2" in comparison to 13 cattle out of 63 animals. For the long known highly prevalent type "O", none and only less than 10% of examined goats and sheep respectively were positive in comparison to more than 60% of examined cattle. Besides, differences between cattle and small ruminants were also observable in titres of positive sera. None of type "O" positive sheep sera showed a titre above 102.1 while positive cattle sera showed titres as high as 103.

Results presented in table (3) show how prevalence to type "O" was very low in the local breeds in comparison to the cross breeds unlike the case in detected type "SAT2" seroprevalence. In table (4) it is evident that seroprevalence of type "O" antibody in tested sheep and type "SAT2" antibody in tested cattle were higher in Western Sudan than Central Sudan. Yet, seroprevalence of type "O" antibody in tested cattle was markedly lower

in Western Sudan, where cattle are exclusively of local breeds, than in Central Sudan, where more than halve of the tested cattle were of the cross breeds.

Discussion

In general, serological tests that detect antibody against structural proteins (SP) of FMD virus are the SN test, the solid-phase competition ELISA (SPCE) and the LPBE. The SN test is advantageous in that it is used to confirm ELISA positive results (OIE, 2008). It was used to confirm absence of antibodies to FMD in free-ranging Roe Deer from Germany whiles the LPBE and the SPCE detected 5.4% and 11.7% prevalence´s respectively (Susan mouchantat et al, 2005). Occurrence of low titre false-positive reactors is not uncommon in both ELISAs (OIE, 2008). Accordingly, establishing of the SN technique for screening of Sudanese livestock is a valuable addition, beside ELISAs, to our armamentarium of FMD serological tests.

In comparison to ELISAs which are easier to perform, the SN test requires a live virus, is laborious in screening of large numbers of sera and makes heavy demand in cell culture services (Golding 1976). The use of a live virus will pose no special problem in the enzootic area of Sudan. In addition, the presented screening format tests more sera in one plate; 20 to 24 sera are tested at two dilutions in one plate, in comparison to 3 or maximum 4 sera when full serum dilution is performed. Results were highly reproducible and no discrepancy that could be related to serum toxicity, virus dose or between the two employed serum dilutions was observed. The viral material used in the survey ("O" and "SAT2") was stable under the described condition of storage. It was adapted to grow in cultured cells through 12 to 16 passages. The character of adaptation was defined as that growth which effect absolute determination of titre. Perhaps this character of definite determination of titre was one reason for the high reproducibility of the assay.

It always raises concern that viruses of FMD would undergo mutational changes in SP after prolonged passages in cultured cells. On the other hand, one condition for sensitivity of FMD serological surveys is the relatedness of the used viral materials to circulating field viruses (OIE, 2008). The screening format detected prevalence's of 82% and 43% in one test group for types "O" and "SAT2" respectively (Table 1) similar to the high prevalence's detected recently by the sensitive LPBE (Habiela *et al,* 2010; Raouf *et al,* 2011).

Using the SN technique, results showed that current infection of type "O" and "SAT2" viruses in Sudan (Habiela *et al,* 2010; Raouf et al, 2010) produced no seroconversion in surveyed indigenous goats and only 9.1% and 1.6% seroconversion in tested sheep sera in comparison to 63% and 20.6% in cattle respectively (Table 2). Titration of positive sera showed, further, the lower titres of positive sheep sera in comparison to titres detected in cattle which could be indicative of the mild nature of infection in sheep. These findings were in agreement with field reports in Sudan that described no overt FMD in small ruminants and with reports that were deprived of isolation of FMD virus from sheep or goats (Abu Elzein, 1983; Habiela *et al,* 2010; Raouf *et al,* 2010). Unapparent FMD infection in sheep and goats in Sudan seems occurring secondary to infection in cattle and no separate cycle of infection could be envisaged in these species as previously suggested (Abu Elzein *et al,* 1987). Recent serological data in the country using the LPBE (Habiela et al, 2010) indicated that FMD infection rates (prevalence's) in sheep and goats also followed the same order that in cattle but at much lower frequency. Prevalence's detected for type "SAT2" were 44% in cattle, 8.9% in sheep, 2.3% in goats and for type "O" were 69.3% in cattle, 27.5% in sheep and goats (Habiela et al, 2010). It is generally accepted that about half of small ruminant flocks are either refractory to clinical FMD or shows only one lesion (Gibson *et al,* 1984; Hughes *et al,* 2002). It would not be unwise to expect that this proportion is even higher in indigenous breeds of sheep and goats in Africa. Moreover, such mildly or unapparent infected animals are likely to excrete small amount of FMD virus in an environment already contaminated with larger amount excreted from cattle what limit the role of sheep and goats in the epidemiology

Table 1: Highest prevalence's detected in some test groups

Type of survey	Volume of test group	No. positive	No. negative	% positive
O	46	38	8	82.6%
SAT2	16	7	9	43.7%

Table 2: Prevalences in cattle and small ruminants

Type of survey	O			SAT2		
Species	Cattle	Sheep	Goats	Cattle	Sheep	Goats
No. tested	95	120	35	63	59	27
No. positive	60	11	-	13	1	-
No. negative	35	109	35	50	58	27
% positive	63.15%	9.16%	-	20.63%	1.69%	-

Table 3: Natural resistance to type "O" in local breeds of cattle

Type of survey	O			SAT2		
Breed	Local	Cross	Total no.	Local	Cross	Total no.
No. tested	30	19	49	30	19	49
No. positive	10	12	22	8	3	11
No. negative	20	7	27	22	16	38
%positive	33.3%	63.15%	44.9%	26.6%	15.79%	22.45%

Table 4: Comparison between detected prevalence's in Western (local cattle breeds) and Central Sudan (cross and local breeds)

Location	Western Sudan			Central Sudan		
Animal species	Local cattle		Sheep	Cattle		Sheep
No. tested	20	48	19	20		
Type of test	"O"	"SAT2"	"O"	"O"	"SAT2"	"O"
% positive	25%	40%	12.5%	73.68%	15.79%	5%

of FMD in Sudan and Africa. On the other hand, change of epidemiological setup that includes highly susceptible animal breeds and climate should be expected to amplify the role of small ruminants in the epidemiology of FMD. Even inside Sudan, in Khartoum state, where many cattle and goats are of the cross and exotic breeds and screened animals were all kept in close contact, using the LPBE higher prevalence's were observed in small ruminants; higher in exotic goats than in sheep. Prevalence's detected for type "SAT2" were 65.78% in cattle, 9.57% in sheep, 21.1% in exotic goats and for type "O" were 81% in cattle, 35.1% in sheep and 49.1% in exotic goats (Raouf *et al,* 2011; Habiela *et al,* accepted for publication).

Using the LPBE to survey cattle in Khartoum state, it was evident that prevalence of type "O" antibody was markedly lower in local breeds than in cross breeds. Prevalence of type "O" antibody, unlike types "A" and "SAT2", decreased markedly at sites where tested cattle were exclusively of the local breeds or where cross and local cattle breeds were reared together. At one site, it was even lower than the medium prevalent type "SAT2" (Raouf *et al,* 2011). Similarly, in this work using the SN test; it was evident that the detected prevalence of type "O" antibody in cattle, unlike that of type "SAT2", was strikingly lower in local breeds (33%) than in cross breeds (63%) (Table 3). In Western Sudan where reared cattle are exclusively of local breeds, prevalence of type "O" antibody in cattle (25%) was even lower than that of the medium prevalent type "SAT2" (40%) (Table 4). Moreover, the known direction

of FMD infection in Sudan is from west and south to east and north following movement of animals (Abu Elzein, 1983). Coinciding with this direction of infection, prevalence of type "O" antibody in sheep, a more resistant species, was higher in Western Sudan (Table 4) than the detected general prevalence in this species (Table 2) and than the detected prevalence in sheep in Central Sudan (Table 4). On the other hand, prevalence of type "O" antibody in cattle in Central Sudan (73.6%), where most of the examined cattle were of the cross breeds, surpassed that in cattle in Western Sudan (25%) (Table 4), where local cattle breeds prevail. These findings are strongly suggestive of resistance of local cattle breeds to serotype "O", apart from other serotypes as demonstrated in this work by type "SAT2". Type "O" was the first FMD virus to be reported in Sudan in 1938, most frequent and most widespread (Abu Elzein, 1983). In comparison, type "A" was first reported in the country in 1957 (Abu Elzein, 1983) while type "SAT2" was reported recently in 1977 (Abu Elzein and Crowther, 1979). No control measures have been applied in Sudan ever since. The observed natural resistance in the local breeds to type "O" seems to coincide with the features of viral epizootics and enzootics where passage in particular species enhance virulence to that species then both virus and host coevolute toward a more symbiotic relationship.

Impact

A feature of FMD is the wide range of animal species that contracts natural infection. Disease intensity differs in these species and within breeds in the same species. One useful approach for studying these varieties is serological surveys. In this study, the SN test, the golden standard, revealed that prevalence's of antibodies against types "O" and "SAT2" were significantly higher in Sudanese cattle than in small ruminants and that of type "O", apart from type SAT2, was significantly lower in local cattle breeds than in cross cattle breeds. Results, confirmed recent findings obtained using the sensitive LPBE. It suggested a diminished role for sheep and goats within the epidemiological setup of FMD in Sudan and development of natural resistance in local cattle breeds to the country longest known FMD type "O" infection.

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COMPARATIVE DISEASE RESISTANCE TO NEWCASTLE DISEASE IN NIGERIAN LOCAL ECOTYPE CHICKENS: PROBABLE GENETIC INFLUENCE

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Abstract

A study was conducted to determine the genetic resistance of two Nigerian ecotypes chicken and exotic breed cockerel (black Nera) to Newcastle disease by evaluating their clinical, haematological and humoral responses to experimental Newcastle disease virus infection..The chicks from the three genotypes were infected with 1ml of 10^5 ELD_{50} of Hertz 33 Newcastle disease virus inoculums orally. Their responses to infection were monitored through clinical signs and mortality, haematological parameters and antibody titres values on days 0, 3, 7, 14 and 21 post infection. There was no adverse clinical manifestation and mortality in all the chicks throughout the experimental period. There was also no significant difference ($p>0.05$) between the mean packed cell volume of all the three genotypes from day 0 up to day 21 post infection though Fulani ecotypes had the highest value at day 21. Lymphocyte counts did not show any significant difference ($p>0.05$) on day 0 and 3 but there was significant difference ($p<0.05$) in the count from day 7 to 21 with Yoruba ecotypes having the highest count. The antibody titres of exotic breed was significantly higher ($p<0.05$) than that of the local ecotypes on day 0) but on day 3, 7, 14 and 21 Yoruba ecotype antibody titres was significantly higher ($p<0.05$) than the other two genotypes. Decrease was observed in the antibody titres level of all the genotypes on day 21 with exotic breed having the least value. From this study, it was shown that Yoruba ecotype chicken had higher immune response to Newcastle disease virus than Fulani ecotype and exotic breed and that the mean antibody titre of $log_2 1.5$ and $log_2 2$ provided protection to the chicks against Newcastle disease as none of the infected chicks show clinical signs and died. Furthermore, it was safely assumed based on the results that Yoruba ecotype chickens are early responder to Newcastle disease and hence are more resistant to Newcastle disease than the other two genotypes.

Key words: Newcastle disease virus, Immune response, Nigerian indigenous ecotypes, chickens

RÉSISTANCE COMPAREE A LA MALADIE DE NEWCASTLE CHEZ POULETS LOCAUX AU NIGERIA: INFLUENCE PROBABLE DE LA GÉNÉTIQUE

Résumé

Une étude a été menée afin de déterminer la résistance génétique de deux écotypes de poulet nigérian et coq race exotique (noir Nera) pour la maladie de Newcastle en évaluant leurs réponses cliniques, hématologiques et humorale à l'infection expérimentale au virus de Newcastle. Les poussins en provenance des trois génotypes ont été infectés par 1ml de 10^5 DLE_{50} des inoculums Hertz Newcastle 33 par voie orale. Leurs réponses à l'infection ont été suivies par des signes cliniques et la mortalité, les paramètres hématologiques et titres d'anticorps aux jours 0, 3, 7, 14 et 21 après l'infection. Il n'y avait pas de manifestation clinique indésirable et de la mortalité chez tous les poussins tout au long de la période expérimentale. Il n'y avait pas de différence significative ($p> 0,05$) entre l'hématocrite moyen de l'ensemble des trois génotypes du jour 0 jusqu'à 21 jours après l'infection bien que l'écotype Peuls ait la plus grande valeur au jour 21. Numération lymphocytaire n'a pas montré de différence significative ($p> 0,05$) au jour 0 et 3, mais il y avait une différence significative ($p <0,05$) dans le décompte aux jours 7 à 21 l'écotype Yoruba ayant le nombre le plus élevé. Les titres d'anticorps de race exotique étaient significativement plus élevés ($p <0,05$) que ceux des écotypes locaux le jour 0), mais aux jours 3, 7, 14 et 21 titres d'anticorps chez les Yoruba étaient significativement plus élevées ($p <0,05$) que chez les deux autres génotypes. De cette étude, il a été montré que le poulet d'écotype Yoruba avait une réponse immunitaire au virus de la maladie de Newcastle plus élevée que ceux de l'écotype Peuls et la race exotique et que les titres moyens d'anticorps de $log_2 1.5$ et $log_2 2$ fournissent une protection pour les poussins contre la maladie de Newcastle.

Mots clés: virus de la maladie de Newcastle, la réponse immunitaire, poulets, écotypes, nigérians

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Introduction

The local chicken is comprised of many phenotypes and genotypes of the species Gallus gallus domestics. There is however a variety of phenotypes characterised by differences in plumage type, distribution and colour, adult body weight, skin colour, egg colour, weight and production (Msoffe *et al.,* 1998,). Local chicken has remained largely genetically uncharacterised and unimproved (Oluyemi and Roberts, 2000). Therefore NRC (1993) recommended a study of the level of genetic diversity in different populations as the first step to bring about improvement in the performance of chicken in the developing countries.

Local chickens had been known for their adaptation superiority in terms of their resistance to endemic diseases and other harsh environmental conditions (Nwakpu et al., 1999). One of the major contributions to this resistance trait is the Major Histocompatibility Complex (B complex) a genetic system, which has been reported to control genetic disease resistance (Bacon 1987). Resistance to Marek's disease by B21 haplotypes, Fowl cholera by B1haplotypes and coccidiosis by B3 haplotypes of White Leghorn chickens have been reported (Briles *et al.,* 1982) while haplotypes associated with other important poultry disease such as Newcastle disease in Nigeria is yet to be determined.

Disease resistance is a trait controlled by multiple genes as well as interactions between several factors (Hartmann, 1997).
Low genetic potential and high prevalence of diseases are among of the major factors limiting productivity of the local chickens in the tropics (Yongolo, 1996). Newcastle disease (ND) has been reported to be the most prevalence (Rahman *et al.,* 2002). It occurs worldwide in poultry of all ages. It is very common disease and one of the most common respiratory diseases of poultry. Spread is airborne by inhalation or by ingestion of the virus. Newcastle disease (ND) is endemic in Nigeria (Saidu *et al.,* 1998). The first documented outbreak of Newcastle disease in Nigeria occurred in Ibadan in 1952 (Hill *et al.,* 1953,). Since then, the disease has been the most important disease of chickens in Nigeria. ND was reported in exotic and local chickens (Abdu *et al.,* 1985). ND continues to be a serious economic threat to the poultry industry resulting in increased morbidity and mortality rates and loss of eggs for both breeding and human. Newcastle disease causes, 60 to 100% mortality of chickens in a household (Yongolo, 1996). With this high mortality, the strategy of mass vaccination has largely been an effective control of the disease, however, combining vaccination programs and development of genetically resistant stocks will further maximize protection of the chickens from the disease.

Despite general believe about resistance of local chicken ecotypes to diseases above their exotic counterpart, few scientific researches have been conducted to establish this fact which makes information about genetic resistance to disease within varieties and ecotypes of local chicken scanty. Local chickens are assumed resistant to infectious diseases but not to all types of diseases as reported by Okoye and Aba-Adulugba (1996). Therefore, there is need to determine possible genetic resistance of local chicken ecotypes to different diseases especially Newcastle disease. The main objective of this study is to assess and compare possible genetic resistance to Newcastle Disease between exotic and different local ecotype chickens.

Materials and Methods

Experimental Site

The experiment was carried out at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The site is located on latitude 7°20'N, 3°50'E, 200m above sea level.

Experimental Chicken

Twenty (20) nine (9) weeks old chicks each from Fulani smooth feathers (FSF), Yoruba Smooth feathers (YSF), and Exotic Breed (EB) - Black Nera Cockerel were used for this experiment. Black Nera Cockerels were purchased at day old and were raised up to four (4) weeks old after which four weeks old Fulani Smooth Feather and Yoruba Smooth Feather chicks were purchased from Fulani Villages at Ilorin and

Villages in Ogbomoso respectively. They were then raised together up to nine (9) weeks old when they were inoculated. The Exotic Breed chicks had history of vaccination against Newcastle disease at day old while the local ecotypes chicks had no history of vaccination. On arrival, the chicks were given antibiotic and Amprolium (anticoccidal) in accordance with the manufactures' recommendations. The chicks were given water and commercial chick mash containing 20% crude protein and 2800ME Kcal/Kg ad-libitum.

Experimental Design

Completely randomized designed (CRD) was used. The birds were allocated into three (3) treatments of Fulani smooth feathers (FSF), Yoruba Smooth feathers (YSF), and Exotic Breed (EB). Each treatment consists of five (5) replicates of four (4) birds per each replicate.

Inoculation

Twenty (20) chicks each from Fulani smooth feathers (FSF), Yoruba Smooth feathers (YSF), and Exotic Breed (EB) were inoculated with 1ml of the Hertz 33 NDV inoculum at 105 ELD50 pathogenic homogenized Newcastle disease virus gotten from National Veterinary Research Institute, Vom. Inoculation was done as ocular and nasal drops, with one drop on each location on one side of the face, while the remainder of the inoculum was given orally.

Parameters Evaluated

Clinical signs and mortality

All the chicks were observed twice daily for clinical signs and mortality up to 15 days post infection and the signs observed and mortality were recorded on daily basis. Clinical sign was presented by scoring with negative sign (-) indicating no clinical signs.

Blood sampling

Before infection and on day 3, 7, 14 and 21 post infection, blood samples were collected from each bird into two bottles one containing Ethylene-diamine-tetra-acetic acid (EDTA coagulant) for Newcastle disease antibodies titre determination, while the other set was without anticoagulant for Packed Cell Volume and differential white blood count (lymphocytes and heterophils).

Determination of packed cell volume

Haematocrit centrifuge technique was used to determine the packed cell volume (Schalm *et al.,* 1975).

Enumeration of selected leukocytes

A thin smear from the blood sample collected was made for leukocyte enumeration. The thin blood films made were air-dried and fixed on absolute methanol for 30 sec. and later stained with Wright's staining method. The films were observed under light microscope(x 1000 magnification) and the cells (lymphocytes and monocytes) were enumerated according to their morphology (100 cells were counted on each slide). The cells were counted and the results were expressed as the percentage distribution (% pd).

Determination of Newcastle disease antibody titres

Haemagglutination Inhibition (HI) test was used to determine specific antibodies against Newcastle disease virus and the method described by Lancaster and Alexander, (1975) was used.

Statistical Analysis

All data collected from the experiment were analysed using one way analysis of variance procedure of Statistical Analytical System (SAS, 1990).

$Y_{ij} = \mu + \propto i + eij$

Where Y_{ij} = Individual observation assumed to be random elements

μ = Population means, fixed

$\propto i$ = Treatment effect, assumed fixed to be determined

e_{ij} = error associated with each record, assumed random and normally distributed

Results

Clinical Signs and Mortalities

There was no clinical sign and mortality observed in all the three groups of chicken throughout the experimental period. This shows that there was no adverse clinical

manifestation or mortalities in the three genotypes of chicken used for this experiment.

Packed Cell Volume

The mean packed cell volume of Yoruba Smooth Feather chicks (28.50%) was slightly higher than that of Fulani Smooth Feather ecotype (27.00%) and the Exotic Breed chicks (26.50%) at day 0 post infection (table 1). At day 3, the mean packed cell volume of Yoruba Smooth Feather chicks was still higher than the other two genotypes but there was no significant difference ($p > 0.05$). At day 7, the mean packed cell volume of Fulani Smooth Feather Chicks (29.25%) was significantly higher ($p < 0.05$) than the mean packed cell volume of Yoruba Smooth Feather Chicks (27.25%) but not significantly different ($p > 0.05$) from that of Exotic Breed chicks (29.00%). On day 14 Fulani Smooth Feather Chicks still had the highest packed cell volume mean (29.50) though it was not significantly different ($p > 0.05$) from the mean packed cell volume of other two genotypes and the same trend continues on till day 21 in which Fulani Smooth Feather Chicks had the highest mean (30.00) and the Exotic Breed with the lowest mean (29.00).

Lymphocytes Count

Table 2 shows the percentage lymphocytes count of the three genotypes of chicken used in this experiment. At day 0 there was no significant difference ($p > 0.05$) between the lymphocytes count of the three groups of chicken used though the Exotic Breed cockerels had the highest mean count (67.00%) while Fulani Smooth Feather chicks had the least mean count (66.75%). The same trend follows up to day 3 with the exception that Yoruba Smooth Feather chicks had the highest mean lymphocytes count (69.50%) with Fulani Smooth Feather chicks having the least mean count (66.75%). On day 7, there was significant difference ($p < 0.05$) between the lymphocytes count of the three genotypes used with Yoruba Smooth Feather chicks having the highest Count (69.75%). There was rise in lymphocytes count of the three group of chicken used in this experiment from day 7 post infection up to day 14 and lymphocytes count declined on day 21 leaving Yoruba Smooth Feather chicks with the highest mean count..

Heterophils Count

Table 3 shows the mean heterophils count of all the genotypes of chicken used. There was no significant difference ($p > 0.05$) between the mean of all the different groups of chicken used in this experiment throughout the experimental period. At day 0 Fulani smooth feather chicks had the highest heterophils count and this trend continued up to day 21 post infection. Decrease in heterophils count was observed in all the three genotypes of chicken from day 0 up to day 14 but slight increase was noticed on day 21

Haemagglutination Inhibition Antibodies Titres

The mean antibodies titre values in $\log_2$ of all the three genotypes of chicken used in this experiment from day 0 up to day 21 post infection were shown in table 6 below. At day 0 , there was significant difference ($p < 0.05$) in the antibodies titre level between the three genotypes with the Exotic Breed having the highest value of $\log_{23}$ and Fulani Smooth Feather chicks having the least value of $\log_2 1.5$. There was increase in the level of antibodies in the chicks throughout the experimental period except on day 21 where decrease in the level of antibodies was observed in all the three groups. At day 3, mean antibodies titre of Yoruba Smooth Feather chicks was the highest ($\log_2$ 5.25) and it was significantly different ($p < 0.05$) from the mean antibodies titre of Fulani Smooth Feather chicks ($\log_2 3.5$) but not statistically different ($p > 0.05$) from that of Exotic Breed chicks ($\log_2 4.5$). At day 7, Yoruba Smooth Feather chicks still had the highest antibodies titre ($\log_2 8.75$) and it was significantly different from the mean of other two genotypes of chicken. This trend continues up till day 14 with Yoruba Smooth Feather chicks having the highest value ($\log_2 9.50$) followed by Exotic Breed chicks ($\log_2 8.75$) and Fulani Smooth Feather chicks with the least value ($\log_2 7.00$). Also at day 21, all the three genotypes had their antibodies level dropped yet Yoruba Smooth Feather chicks still had the highest value. The rate of fall was higher in Exotic Breed chicks than that of Fulani Smooth

Table 1: Packed Cell Volume (%) of the chicks

	Infected chicks			
Days (post infection)	SEM	FSF	YSF	EB
0	27.00	28.50	26.50	0.39
3	27.50	29.00	27.75	0.38
7	29.25[a]	27.25[b]	29.00[a]	0.31
14	29.50	27.50	28.75	0.46
21	30.00	29.25	29.00	0.49

Means in the same row with different superscripts are significantly (P<0.05) different.

Table 2: Lymphocyte count (%) of the chicks

	Infected chicks			
Days (post infection)	SEM	FSF	YSF	EB
0	62.75	65.75	67.00	1.46
3	66.75	69.50	68.25	1.16
7	69.75[b]	74.50[a]	70.25[b]	0.48
14	71.50[b]	77.25[a]	72.50[b]	0.48
21	70.00[b]	76.25[a]	71.25[b]	0.50

Means in the same row with different superscripts are significantly (P<0.05) different.

Table 3: Heterophils count (%) of the chicks

	Infected chicks			
Days (post infection)	SEM	FSF	YSF	EB
0	28.50	26.25	26.00	1.47
3	24.50	22.50	24.50	1.20
7	22.75	19.00	23.25	1.14
14	21.50	15.25	19.50	1.00
21	22.00	16.25	21.75	1.10

Means in the same row with different superscripts are significantly (P<0.05) different from each other.

Feather chicks which make the Exotic Breed chicks to have the least antibodies titre value (log_2 6).

Discussion

The absence of clinical signs and mortalities observed in all the three groups of chicken used in this study was contrary to the findings of Msoffe *et al.* (2002) who reported that there was clinical signs and mortalities in all chickens experimentally infected with Newcastle virus starting from three (3) days post infection. This discordant could be partly due to either variation in dose or strain of virus used. It could also be probably due to presence of specific antibody found in the chicken prior to infection though at a low level might have protected the chicks clinically. Nevertheless, the result of this present study supported the work of Rwuaan (2009) who noticed that chickens with low level of antibody titre below protective level of $log_2 3$ as reported by Allan and Gough (1974) when infected with Newcastle virus showed no clinical signs and mortalities. Therefore, it was seen clearly from this work that chickens with antibody titre below $log_2 3$ could be protected against Newcastle infection possibly due to genetic make up of the local chickens as the exotic breed used had protected antibody titre ($log_2 3$) prior infection, hence the protection observed.

The mean PCV of all the three genotypes of chicken used in this experiment was lower than the normal physiological range

Table 4: Haemagglutination Inhibition Antibody Titres (Log2) of the chicks

	Infected chicks			
Days (post infection)	SEM	FSF	YSF	EB
0	1.50[b]	2.00[a]	3.00[a]	0.22
3	3.50[b]	5.25[a]	4.50[a]	0.21
7	5.75[b]	8.75[a]	6.00[b]	0.23
14	7.00[b]	9.50[a]	8.75[a]	0.19
21	6.50[b]	8.75[a]	6.00b	0.33

Means in the same row with different superscripts are significantly (P<0.05) different.
Where FSF = Fulani smooth feathers,
YSF = Yoruba Smooth feathers
EB = Exotic Breed
SEM: Standard error of mean

(Ikhimioya *et al.,* 2000). The low in PCV could be probably due to the differences in chicken used or the age of chicken used for the study. There was no significant decrease in the mean PCV of all the three groups of chicken post infection throughout the three-week period of observation. This was contrary to the observation of Oladele (2004) who reported a decrease in the PCV two days post infection and which continued up to day fourteen (14) but agrees with the findings of Rwuaan (2009) who reported no decrease in the mean PCV of the chicken post infection throughout the six weeks of experiment. The reason for this could probably be that the chicken used in this study had specific antibody against Newcastle disease at day zero (0) though at a level below protective level which prevented the clinical form of the disease as the immunity against Newcastle infection prevented haem-agglutination by the virus.

The increase in the level of lymphocytes count in all the three groups of chicken used from day zero (0) up to day fourteen (14) post infection and the decrease observed on day twenty one (21), could partly due to the fact that lymphocyte is one of the white blood cells that are involve in body's immune response to viral infection. It could also be due to the function of lymphocytes in the production of antibody.

The result presented in this study fall within the normal physiological range (Dukes, 1955). This finding is in agreement with the finding of Sturkie (1965) but contradict the work of Oyewale (1987) who reported that Nigeria local fowls had lower lymphocytes count. Before infection, exotic cockerels had the highest lymphocytes been the group of chicken with highest antibody titre, but on at day three (3) post infection Yoruba ecotype had greater response to the infection and had more lymphocytes than the other ecotypes. The reason for this is not clear but it could be partly due genetic which might have aided the recognition of the pathogen faster. Lymphocytes count peaked on day fourteen post infection in all the three groups of chicken and this coincide with antibody titre peak which suggest that their might be positive correlation between lymphocytes count and antibody titres in infected chickens.

Heterophils are granulocytes which are the first line of cellular defense towards invading pathogens (Toth and Siegel, 1986). From the result of this study, the percentage heterophils reported is in agreement with the work of Islam (2004).

From table 5 above, it was seen that both group of local ecotype chicken used had specific antibody at a level below protective level. The reason for this might be partly due to the fact that those chicks had been exposed to subclinical field strain of Newcastle virus and responded by producing antibody which support the finding of Nwankiti (2010) who observed that some level of antibodies were found in local chicken who had no history of vaccination. The present of antibody in the exotic breed at day zero (0) confirmed that they had maternal antibodies at day old. The present of specific antibody against Newcastle

disease be it response or maternal might be the reason all the three groups of chick did not come down with the disease which support the work of Marino and Hanson (1987) who reported that chicken with antibody titre do survive against Newcastle disease virus infection. Chickens with antibody titres below log23 may also be protected probably by cell mediated or mucosal immunity (Sofus Bogi, 2003), this might suggest the involvement of cell mediated immunity in chickens during infection of Newcastle disease. The inoculation of the chicks resulted into seroconversion from day three (3) post infection up to day fourteen which increased the antibody titres of the chicks. This indicated that the chicks developed a stronger protection as high antibody titre was observed. Furthermore, the result of this study also agrees with the finding of Oladele, (2004) who infected Brown shaver birds with Newcastle virus and reported that antibody titre increased by day two (2) post infection. Decrease in antibody titre observed on day twenty (21) in all the three genotypes also agrees with the work of Msoffe (2006) who reported decrease in antibody titre at day twenty eight (28) post vaccination in local chickens. The antibody titers peaked in all the three genotypes of chicken used at fourteen (14) day post infection which is in accordance with the work of Sofus Bogi (2003) who reported peak in antibody titre in experimentally infected chicken at two weeks post infection.

When the local chicken ecotypes and exotic breed were compared on their responses to Newcastle disease virus, there were significant differences in the mean antibody titre from day three (3) up to day twenty one (21) post infection. Yoruba ecotype as at day three (3) post infection had highest antibody titre than exotic breed which had highest titre at day zero (0). This suggests that Yoruba ecotype responded faster to Newcastle infection than Fulani ecotype and exotic breed and early response to infection is one of the factors that determine genetic resistance to infection and it is also an advantage when it comes to survival during Newcastle disease outbreak (Msoffe, 2006). Mtambo (1999) reported that high level of antibody titre contributed towards the recovery of infected birds. From the result of this study, it was seen that Yoruba ecotype is an early responder to Newcastle disease infection than both Fulani ecotype and exotic breed. The reason for this might be genetic which helps Yoruba ecotype to detect the virus early and responded faster than the other genotypes. The genetic system known to involve in resistance to infection is the major Histocompatibility complex which help the immune system to recognise pathogen and present them to the cell mediated immunity which will later present them to humoral immune system. This early response to Newcastle disease virus in Yoruba ecotype might be the reason the chicken survive in their scavenging environment without vaccination and yet develop some level of antibody which protect them from Newcastle disease infection. At day twenty one (21) post infection when decrease was noticed in the antibody titre of all the three genotypes, Fulani ecotype had slight drop in antibody titre compare to exotic breed. The reason for this might be probably due to the fact that the Fulani ecotype chicken is believed by some authors (Ogundipe, 1990; Tiamiyu, 1999) to have been a crossbreed between indigenous fowls and the Rhodes Island Red (RIR) chicken which suggests that they may perform better than the exotic breed in terms of disease resistance.

Conclusion

The results of this study indicates that indigenous chicken with antibody titre value less than $\log_2 3$ did not come down clinically when infected with Newcastle disease virus. It is therefore suggested that birds with antibody titre less than $\log_2 3$ may be protected when exposed to Newcastle disease virus. This study also confirmed that humoral immune system is a key component in protection of chicken against Newcastle disease as increase in the level of antibody led to protection of chicken against the infection.

The findings from this present study showed that there was significant difference in the level of antibody produced between the three genotypes of chicken used. Yoruba ecotype responded faster than other two

groups of chicken by producing high level of antibody throughout the experiment. Based on the results, it is therefore suggested that the observed differences in response to Newcastle disease infection between local chicken and exotic breed are genetic and it could be utilized in selective breeding programme both between and within ecotypes. Furthermore, based on the results of this study it can therefore be safely assumed that Yoruba ecotype chicken is more resistant to Newcastle disease than the Fulani ecotype and the exotic breed. Hence it is therefore imperative to establish the link between fast response potential to infection in local chicken ecotypes with productive traits such as growth rate, body weight, egg size.

Research into major histocompatibility complex genes responsible for resistance to disease should be embarked on.

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SHORT COMMUNICATION

EVALUATION DES PERTES EN VEAUX PAR ABATTAGE DES FEMELLES GRAVIDES A L'ABATTOIR FRIGORIFIQUE DE OUAGADOUGOU

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Le Burkina Faso est un pays à vocation agro-sylvo-pastorale dont l'économie est basée sur le secteur primaire qui occupe la première place avec 80% de la population active. En dehors de sa fonction de pourvoyeur de devises et des protéines animales, l'élevage joue un rôle important dans la lutte contre la pauvreté au niveau des couches sociales les plus défavorisées de la société (Traoré et al., 2008). L'effectif bovin a été estimé à 7.331.544 têtes toutes races confondues avec un taux de croît de 2% (MRA, 2004). Plus de 80% de l'effectif bovin, est encore élevé selon le mode extensif et reste confronté à de nombreuses contraintes qui occasionnent sa faible productivité (Ba, 2002). Ces contraintes sont nombreuses et multiformes et les plus fréquemment évoquées sont le déficit alimentaire et la forte prévalence des maladies parasitaires et infectieuses entraînant de fortes mortalités parmi les jeunes qui sont plus vulnérables (Doulkoum *et al.,* 2005). En plus de ces contraintes et des maladies abortives, il existe une autre forme de pertes des veaux plus discrète au point de demeurer jusqu'à présent méconnu, mais joue un rôle très important dans la faible productivité générale du troupeau bovin au Burkina Faso. Il s'agit des pertes en veaux par abattage des femelles gravides. Des études menées sur les bovins, les porcs et les petits ruminants au Cameroun (Manjeli *et al.,* 1996 ; Tchoumboué, 1996 ; Tchoumboué, 1998) et au Nigeria (Wosu, 1988) ont relevé respectivement que 16,61% des vaches, 27% des truies, 38,6% des chèvres et 59,2% des brebis examinées, après abattage, étaient gestantes. Des taux similaires de 26,70% chez les brebis et 34,91% chez les chèvres ont été rapportés au Burkina Faso (Traoré *et al.,* 2008). Ces chiffres situent l'importance du manque à gagner par l'abattage des femelles gravides. Par contre, ces études au Burkina Faso n'ont pas concerné les bovins.

L'objectif de la présente étude est d'évaluer l'importance des pertes en veaux par abattage des vaches gestantes à l'Abattoir Frigorifique de Ouagadougou afin d'estimer l'incidence financière inhérente à ces pertes.

L'étude a porté sur la population bovine abattue à l'abattoir frigorifique de Ouagadougou d'août à novembre 2005. Ces animaux proviennent des différents régions et marchés à bétail du Burkina Faso. Au total, 20. 216 bovins ont été abattus durant la période de l'étude dont 13. 776 femelles et 6. 440 mâles.

Le matériel utilisé pour la manipulation comprenait un peson (sensibilité ±0,1) , un couteau d'inspection, une paire de ciseaux, un mètre à ruban, des gants de fouille et un appareil photo numérique pour la prise de vue.

La détermination du nombre de femelles a été réalisée par comptage au cours de l'abattage. Les fœtus collectés ont été repartis en trois (3) classes d'âge : 1– 3 mois ; 3 - 6 mois et 6-9 mois (Nsekayarenze, 1988).

Une évaluation financière a été faite sur la base du prix moyen du veau au marché à bétail de l'AFO et de la nomenclature des catégories de bovins (Drion *et al.,* 1993). Elle a été estimée sur la période du stage et une projection a été faite sur une année.

Le pourcentage de femelles abattues à l'abattoir Frigorifique de Ouagadougou représentait les deux tiers des mâles abattus 68,14% contre 31,86. Ces vaches étaient toutes âgées de 3 à 12 ans et plus mais les vaches de la tranche d'âge de 7 à 12 ans représentaient 71,61% des femelles abattues contre 13,12% des femelles de plus de 12 ans. Sur les 13 776 vaches abattues, 2 538 étaient gestantes soit un

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taux de positivité de 18,42% (tableau).

Lee taux d'abattage des vaches gestantes est de 67,92% pour le premier tiers de gestation 1-3 mois contre 29,59% pour le deuxième tiers de 3-6 mois et seulement 2,48% pour le dernier tiers 6-9 mois (Photo).

Photo : Fœtus âgé de 6 à 9 mois

La perte numérique en veaux consécutive à l'abattage de leur mère est égale au nombre de vaches gestantes abattues soit un total de 2 538 veaux. L'estimation financière a été faite sur la base du prix moyen du veau vendu au marché à bétail de l'AFO. Selon les Services des Statistiques Animales du MRA en 2003, le prix du veau de moins d'un an est de 72 Euros. Ainsi, la perte financière est estimée à 182 466 Euros au cours de la période de notre étude si toutes les gestations arrivaient à terme. En considérant que les mêmes évènements moyens resteront identiques au cours d'une année, la perte financière annuelle est estimée à 729 863 Euros pour le seul Abattoir Frigorifique de Ouagadougou.

Cette étude montre que 68,14% des bovins abattus et contrôlés à l'abattoir frigorifique de Ouagadougou sont des femelles contre 31,86% de mâles. Les femelles sont donc deux fois plus exploitées que les mâles (Simukoko *et al.,* 2007). L'étude montre l'importance des pertes de veaux dues à l'abattage des vaches gestantes (18,42%) au Burkina Faso. . Ce taux est supérieur à celui rapporté au Nigeria (wosu, 1988) et au Cameroun (Tchoumboué, 1988) qui était respectivement de 9,77% et 16,61% chez les vaches. Cette valeur devrait être revue à la hausse puisque la méthode utilisée ne permet pas de prendre en compte les femelles abattues avant la nidation ou la placentation ou à un stade de gestation très précoce (Seibou, 2000).

Le taux d'abattage des vaches gestantes régresse au fur et à mesure que la gestation tend arrive à terme. Dans cette étude, ce taux régresse de 67,92% chez les vaches gestantes de 1-3 mois à 29,59% chez les vaches gestantes de 3-6 mois et 2,48% chez les vaches gestantes de 6-9 mois. Des travaux antérieurs au Cameroun (Manjeli *et al.,* 1996 ; Tchoumboué, 1989, 1988, 1984), au Nigeria (Wosu, 1988) et en Zambie (Simukoko et al., 2007) ont rapporte des valeurs similaires. Par contre, on note une nette régression du taux de vaches abattues à 6-9 mois de gestation de 0,82% à 0,15%. Cela pourrait s'expliquer par la famine qui a amené les éleveurs à vendre les animaux quelque soit leur état physiologique pour subvenir aux besoins de la famille. La période de l'étude pourrait avoir une influence significative sur le taux d'abattages des vaches gestantes. En outre, la période de l'étude (août à novembre) permet une amélioration de l'état physique favorable à la reproduction. Cette situation est aussi imputable à plusieurs facteurs qui sont le système d'élevage, le faible niveau de technicité des producteurs et le non respect de la législation vétérinaire en matière d'abattages des femelles. En effet, au Burkina Faso, le système extensif est largement pratiqué à plus de 80% où les animaux sont conduits ensemble au pâturage. La reproduction n'est pas maîtrisée et se fait au gré de la panmixie (Ouedraogo, 1995).

L'étude a également révélé que les principales causes de l'abattage des femelles sont essentiellement la réforme des vaches, âgées, accidentées, ou souffrant d'une maladie abortive connue telle que la brucellose, la tuberculose, la toxoplasmose mais surtout l'ignorance de l'état physiologique des animaux. L'éleveur ignore l'état physiologique des vaches et les vend sans discernement en fonction de ses contraintes financières (alimentation, santé, scolarité) (Tchoumboué, 1988 ; Wosu, 1988 ; Drion *et al.,* 1993). A cela, il faut ajouter la non inspection sur des animaux sur pied avant l'abattage et la non application des textes réglementaires en la matière (Ndi *et al.,* 1993 ; Wilson *et al.,* 1988).

L'évaluation les pertes en veaux par l'abattage des vaches gravides montre une perte d'environ 729 863 Euros à l'économie nationale par an à l'AFO. Ces chiffres montent un manque à gagner non négligeable qu'il serait nécessaire d'estimer au plan national afin d'envisager des mesures d'urgence (Wosu, 1988 ;Wilson et al., 1993 ; Kulo *et al.,* 2007) .

L'étude montre l'importance des pertes de veaux dues à l'abattage des vaches gestantes à l'abattoir frigorifique de

Tableau 1: Récapitulatif de la répartition des bovins abattus et des vaches gestantes selon l'âge de la gestation

Eléments de récapitulation	**Durée de l'étude (3 mois)**
Nombre d'animaux abattus et contrôlés	20 216
Nombre de mâles abattus et contrôlés	6 440 (31,86%)
Nombre de vaches abattues et Contrôlées(VAC)	13 776 (68,14%)
Nombres de vaches gravides abattues(VGA) Pourcentage par rapport aux VAC	2 538 18,42%
Nombre de vaches abattues à 1-3 mois de gestation Pourcentage par rapport aux VGA	1724 67,92%
Nombre de vaches abattues à 3-6 mois de gestation Pourcentage par rapport aux VGA	751 29,52%
Nombre de vaches gestantes 6-9 mois Pourcentage par rapport aux VGA	63 2,48%

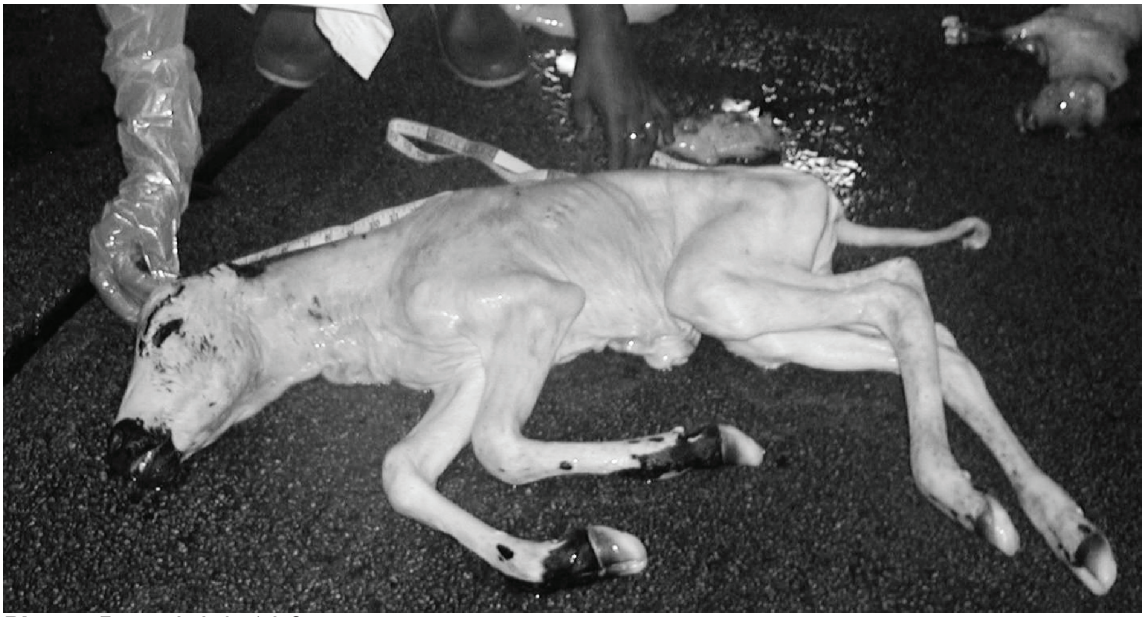

Photo : Fœtus âgé de 6 à 9 mois

Ouagadougou. Elle révèle que le stade de la gestation a une forte influence sur le nombre de femelles abattues. Ce taux élevé de gestantes parmi les femelles abattues est dû d'une part à la période de l'étude, qui s'est déroulée en période de soudure contraignant les éleveurs à vendre leurs animaux pour satisfaire les besoins de la famille mais aussi une période où l'état des animaux se trouve amélioré et de ce fait favorable à la reproduction. D'autre part ce fort taux a des raisons profondes comme le non respect de la législation en matière d'abattage contrôlé, la méconnaissance de l'état physiologique des animaux et le système d'élevage a prédominance extensif.

L'estimation des pertes financières pour la durée de l'étude montre un manque à gagner non négligeable pour l'économie nationale. A l'échelle nationale, il s'agit de pertes économiques importantes contre lesquelles des mesures urgentes doivent être envisagées pour en diminuer l'ampleur. Il s'agit de l'application rigoureuse de la législation vétérinaire en matière d'abattage contrôlé ; la sensibilisation des acteurs sur l'ampleur et l'incidence économique de l'abattage des femelles gestantes ; la formation des agents en techniques simples et moins coûteuses du diagnostic de gestation. Cependant à long terme il faudrait travailler à une modernisation de l'élevage avec des acteurs maîtrisant la reproduction.

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SHORT COMMUNICATION

EFFECT OF ROAD TRANSPORTATION OF CATTLE BETWEEN TRANSBOUNDARY AREA AND CENTRAL ABATTOIR OF ABEOKUTA, OGUN STATE ON PLASMA CORTISOL, BLOOD GLUCOSE AND LEUKOCYTE PARAMETERS

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Road transport stress is the acquired stress condition of animals during their transportation by roads in which the rules and regulations governing normal transport welfare are not adhered to (Minka and Ayo, 2010). Most of the changes in the body parameters of transported animals are as a result of handling, loading and confinement of animals during transportation, as well as, unloading of the animals. Other stress factors include improper restraint of the animals and non-provision of food and water during transportation (Maria *et al.,* 2007). The stress factors occurring during transportation cause physical and physiological exertion which disrupt metabolism of animals. Transportation stress has been shown to cause increase in plasma total protein, blood urea, non-esterified fatty acids, erythrocyte and leukocyte counts, plasma glucose and cortisol concentration (Averos *et al.,* 2008; Ndlovu *et al.,* 2008). The severity of the metabolic changes following transportation stress is dependent on factors such as size and type of vehicle used, duration of journey, sex of the animal, age of the animal, time of transportation and the climatic condition during transportation (Ritter *et al.,* 2008). In Abeokuta, Ogun State, cattle slaughtered are often transported in open, medium- sized trucks over a journey of about one hour from the trans-boundary area to the central abattoir. Transportation is often during the late evening till early hours of the next day. In this study, we evaluated the effect of one hour road transportation of cattle on certain stressor indices such as plasma cortisol, blood glucose and leukocyte indices and compared with the results cattle that were just returned from grazing.

Eighty-two adult white Fulani cattle comprising both sexes were used. The first group of cattle comprised sixty- two animals that were transported over a journey of about one hour from the transboundary area to the lairage of the central abattoir for slaughter. The second group of cattle comprised twenty animals, housed at the Teaching and Research Farm of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. About 5mls of blood was collected from the jugular vein of each cattle using a 5mls syringe and a size 19 gauge needle. The samples were divided equally into ethylene diamine tetra-acetic acid (EDTA) bottle for the determination of plasma cortisol and leukocyte parameters, and fluoride oxalate bottle for the determination of blood glucose. The total white blood cell counts, leukocyte differentials and blood glucose were analyzed using automated blood analyzer (Beckman Coulter, UK). The absolute counts for neutrophils and lymphocytes were calculated from their differentials. Plasma cortisol concentration was determined using the Coat-A-Count Assay kit (Siemens Health Diagnostics, CA, USA), adapted and validated for bovine plasma (Kannan et al., 2001). Each sample was run in duplicate and the mean value obtained. Total binding was 51 percent and the non-

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specific binding was 1.1 percent. Data were expressed as mean (standard deviation) and compared between the two groups of cattle using Student's t-test. All statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Correlation between parameters was evaluated using Pearson's correlation. A value of $P< 0.05$ was considered significant.

The plasma cortisol concentration was significantly ($P< .001$) higher in road-transported cattle (Group A) than non-transported cattle (Group B) (Table 1). Similarly, the blood glucose was significantly ($P< 0.05$) higher in Group A cattle than the Group B cattle. However, there were no significant differences in total white blood cell counts (tWBC), absolute neutrophil counts (NEUT), absolute lymphocyte counts (LYM) and neutrophil lymphocyte ratio (N/L) between Group A and Group B cattle (Table 1). Also, the plasma cortisol concentration and the blood glucose levels were significantly ($P= 0.05$) and positively correlated ($r=0.230$) among the cattle (Table 2). Similarly, tWBC, NEUT, LYM and L/N were significantly and positively correlated. However, there was no correlation between the plasma cortisol concentration and leukocyte parameters among the cattle (Table 2).

The results of this study showed that road transportation of cattle from the transboundary area to the central abattoir in Ogun State over duration of one hour was characterized by stress associated physiological changes such as elevated plasma cortisol and blood glucose. However, there were no significant changes in the leukocyte parameters of the cattle. Also, findings from this study showed that there was positive association between plasma cortisol and the blood glucose concentration further confirming the role of the two biochemical molecules as an important stress biomarker in cattle.

The activity of the adrenal cortex is increased in physiological stress resulting in elevated plasma cortisol. Physiological stress response in animal may be in reaction to physical restraints during handling, transportation of animals or pain associated with surgery or painful husbandry practices such as dehorning or disbudding, tail docking etc. (Faulkner and Weary, 2000). Elevated plasma concentration of cortisol has been reported in cattle following transportation (Odore *et al.,* 2004). In this study, the plasma concentration of cortisol was significantly higher in cattle that were transported in trucks for about one hour compared with those that were not transported but just returned from grazing. This change in the plasma concentration of cortisol may be due to stress associated with improper restraint of the animals. Plasma glucose is commonly used as a physiological indicator of stress. Increased activity of the adrenal cortex in response to stress results in increased production of cortisol which will in-turn activate the breakdown of muscle and liver glycogen into glucose (Tadich *et al.,* 2005). The severity of cortisol –induced breakdown of the body glycogen is thus dependent on the duration of the stressor factor. In this study, the blood glucose concentration was significantly higher in cattle transported for one hour when compared with control cattle that were not transported but just returned from grazing. The positive correlation between plasma cortisol concentration and the blood glucose further support the evidence that the increase in the blood glucose concentration is occasioned by the cortisol-induced break down of glycogen.

Elevation in the plasma cortisol has been shown to suppress immune functions by producing characteristic changes in the population of leukocytes and altering cell mediated immunological functions (Burdick *et al.* 2011). This is characterized by a decrease in the blood lymphocytes and an increase in the blood neutrophils (Minka and Ayo, 2007). In this study, there were no significant changes in the leukocyte indices of the road- transported and non-transported cattle, although the total white cell counts was higher in the non-transported cattle than the road -transported cattle. The short duration of transportation in this study might have accounted for the reason why the leukocyte parameters were not significantly different between the road-transported cattle and the control group.

In conclusion, the result of this study showed that the present method

Table 1: Changes in plasma concentration of cortisol, blood glucose and leukocyte parameters of white Fulani cattle transported on road for one hour and non-transported cattle

Parameters	Cattle	
	Road Transported Cattle (Group A)	Non-Transported Cattle (Group B)
Plasma Cortisol (ng/ml)	53.7 ± 17.6	9.1 ± 3.9**
Blood Glucose (mg/dl)	112.9 ± 13	87 ± 8.3*
Total white blood cell counts (x 10^6/l)	8002 ± 3567	9280 ± 3568
Absolute neutrophil counts (x 10^6/l)	4321 ± 2318	5026 ± 2083
Absolute neutrophil counts (x 10^6/l)	3403 ± 1212	3816 ± 1757
Neutrophil/lymphocyte ratio	1.2 ± 0.28	1.5 ± 0.38

* P< 0.05,

Table 2: Pearson correlation coefficient of selected blood parameters in cattle following one hour transportation

Parameters	CORT	GLU	tWBC	NEUT	LYM	N/L
Plasma cortisol (CORT)						
r	1	.230	.078	.111	.036	.135
p	1	.0508*	.500	.359	.765	.264
Blood Glucose (GLU)						
r	.230	1	-.072	-.038	-.123	.015
p	.050*	1	.556	.756	.312	.902
Total white blood cell counts (tWBC)						
r	.078	-.072	1	.958	.954	.766
p	.520	.556	1	.000*	.000*	.000*
Absolute neutrophil counts (NEUT)						
r	.111	-.038	.985	1	.892	.856
p	.359	.756	.000*	1	.000*	.000*
Absolute lymphocyte counts (LYM)						
r	.036	-.123	.954	.892	1	.553
p	.756	.312	.000*	.000*	1	.000*
Neutrophil-lymphocyte ratio (N/L)						
r	.135	.015	.766	.856	.553	1
p	.264	.264	.000*	.000*	.000*	1

of transportation of cattle from the transboundary area to the lirage at the central abattoir in Ogun State, Nigeria, which involved the use of medium -sized trucks and tying of the cattle with ropes during transportation is stressful to the cattle with the attendant physiological responses such as elevated plasma concentration of cortisol and blood glucose and a tendency towards decrease in immune response of the animal if the journey is prolonged further. This effect if not prevented may exacerbate latent bacterial infections which may adversely affect the carcass quality of the animal after slaughter.

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CASE STUDY REPORT

MANDIBULAR MORPHOLOGICAL CHANGES ASSOCIATED WITH ACTINOMYCESVISCOSUS INFECTION IN A WEST AFRICAN DWARF GOAT IN NIGERIA.

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Abstract

This report described the morphological alterations on the mandible in a 3- year- old West African dwarfdoe caused by Actinomycesviscosus infection. The animal recovered after treatment but was later culled and the head submitted to the Department of Veterinary Anatomy. Hot water maceration of the lower jaw and the histology of the right mandibular lymph nodewere carried out. The mandible revealed worn off periodontal membrane, lodgment of tough feed materials in the gingival-alveolar spaces as well as a fistulous tract of about 4mm in diameter with uneven ridges at its edges ventral to the 2nd molar on the lateral aspect of the horizontal ramus of the mandible. Also, the alveolar borders of both sides of the mandible were worn off with the right side forming a thicker ridge than the left. While the mandibular tuberosity on the median surface of the right mandible was more prominent than the left, there was equally a distortion in the alveolar alignment with deviation towards the median plane. Histologically, the right mandibular lymph node revealed moderate fibroplasia with cortical lymphoid hypoplasia and local area of mineralization with mononuclear cell infiltration (mostly macrophages) in the sinuses. These findings showed a great similarity in the mandibular morphological changes in the West African dwarf goat and other small ruminants irrespective of the causative agent. It also showed that these changes are permanent and could lead to imbalance in the alignment of the upper and the lower jaws thereby impairing chewing and consequently, the growth of the affected and even treated animals.
Keywords: Mandibular changes, Osteomyelitis, West African dwarf goat, Actinomycesviscosus

MODIFICATIONS MORPHOLOGIQUES DE LA MANDIBULE ASSOCIÉES À L'INFECTION ACTINOMYCESVISCOSUS CHEZ UNE CHÈVRE NAINE D'AFRIQUE OCCIDENTALE AU NIGERIA

Resume

Ce rapport décrit les modifications morphologiques de la mandibule chez une chèvre nained'Afrique occidentale de 3 ans, causées par une infection Actinomycesviscosus. L'animal a récupéré après le traitement, mais a été abattu plus tard et sa tête remise au Département d'anatomie vétérinaire. Une macération à l'eau chaude de la mâchoire inférieure et l'histologie du ganglion lymphatique de la mandibule droite ont été effectuées. La mandibule a révélé une membrane parodontaleusée, un dépôt de matières alimentaires solides dans les espaces gingivo-alvéolaires ainsi qu'un trajet fistuleux d'environ 4 mm de diamètre avec des crêtes inégales à ses bords ventraux vers la 2ème molaire sur la face latérale de la branche horizontale de la mandibule. En outre, les bords alvéolaires des deux côtés de la mandibule étaient usés, le côté droit formant une crête plus épaissequele côté gauche. Alors que la tubérosité mandibulaire sur la surface médiane de la mandibule droite était plus importante par rapport au côté gauche, il y avait aussi une distorsion de l'alignement alvéolaire avec une déviation vers le plan médian. Histologiquement, le nœud lymphatique de la mandibule droite a révélé une fibroplasie modérée avec une hypoplasie lymphoïde corticale et une minéralisation de la zone locale avec une infiltration de cellules mononucléaires (surtout des macrophages) dans les sinus. Ces résultats ont montré une grande similitude au niveau des changements morphologiques de la mandibulede la chèvre naine d'Afrique occidentale et d'autres petits ruminants, indépendamment de l'agent étiologique. Ilsont également montré que ces modifications sont permanentes et sont susceptibles decauser un déséquilibre dans l'alignement des mâchoire, supérieure

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inférieure,conduisant à une incapacitéde mâcher et, partant, freinantla croissance des animaux atteints même s'ils sont traités.

Mots-clés : Modifications de la mandibule, Ostéomyélite, Chèvre naine d'Afrique occidentale, Actinomycesviscosus

Introduction

Chronic Osteomyelitis is a relapsing and persisting infection that evolves over months to years and is characterized by low grade infection, presence of bone sequestra, new bone apposition, and some times fistulous tract (Zuluaga, *et al.,* 2006).In maxillo-facial skeleton, chronic osteomyelitis is more often observed in the mandibular and maxilla where it can either be limited to a unique anatomic site or spread to other areas (Brady, *et al.,* 2006).

Mandibular osteomyelitisreported frequently in animals is mostly caused by bacterial infections and is often predisposed by previous trauma (Thompson, 2007). The most commonly involved bacteria are Actinomycesspp, *Arcanobacteriumpyogenes, Escherichia coli, Pseudomonas,Salmonella* and *Staphylococcus species* (McGavinet *et al.,* 2001;Thompson, 2007; Benito- Pena *et al.,* 2010).The Actinomycesspp. that are most commonly incriminated in this infection in domestic animals includes *A. bovis, A. hordeovulneris, A. isrealii, A. naeslandii, A. pyogenes, A. suis* and *A. viscosus*(Kahn, 2005). Though caused by *Actinomyces species* , and occasionally by other species, the morphological changes on the mandible differ from one animal host to another as well as the causative agents.

In man, *A. isrealii* was reported to cause *cortical osteomyelitis* of the ascending ramus of the mandible (Gupta *et al.,* 1985), while inA. viscosus, *Fusobacterimnucleatum* and *Bacteroides* spp infections in dogs and cat, acircumscribed zone of cortical bone lysis, sequestra and periosteal new bone formation were demonstrated on the body of the left mandible (Johnson *et al.,* 1984).In cattle, the manifestation of *A. bovis* infection on the mandible is a marked increase in the bone density as well as osteolytic foci at the mandibular cortex with the affected bone much thicker than the normal one (Bargai *et al.,*1989). In sheep, mandibular osteomyelitis is reported to cause deformed bone structure with extensive necrotic area within the trabecular bone (Benito- Pena *et al.,* 2010).

Generally, bacterial *osteomyelitis* is not common in goats(Seifi *et al.,* 2003). However, while Hirai *et al.,* (2007) reported that *A. naeslandii* causes multifocal abscess of the temporal bone, Seifi *et al.,* (2003) documented a case of mandibular *osteomyelitis* caused by *Arcanobacteriumpyogenes* in goat. The present report highlights the morphological alterations associated with the mandible, in a rare case of *Actinomycesviscosus* infection in a West African Dwarf (WAD) doe in Nigeria.

Materials and Methods

Case History

A 3-year old female West African Dwarf (WAD) goat with the body mass of 15kg, fromtheTeaching and Research Farm was presented to the Veterinary Teaching Hospital, University of Agriculture Abeokuta, Nigeria with a cutaneous swellingof the right mandible which was painful on palpation. History indicated a gradually advancing, painless, hard and immovable swelling which later became painful to touch after about 3 weeks.The clinical and bacteriological diagnosis of the condition as Actinomycosis caused by Actinomycesviscosus and its medical management were previously discussed (Oyekunle *et al.,* 2010).

Dissection and Preparation of the mandible: The animal was later culled and the head submitted to the Department of Veterinary Anatomy for dissection. The mandible was dissected out and de-articulated from the skull at the temporo-mandibular junction. The remaining soft tissue removed from the mandible by hot water maceration according to the method described by Olopade and Onwuka (2005). Briefly, the method entails heating the lower jaw in a solution of Polycarboxylate and anionic surfactant (i.e. detergent and soap chips) over 100°C for 1-2hours.The jaw was then put under running water and muscleson the bone,which has became so tender, was easily separated form it

with the aid of forceps without any damage to the bone. The bone was then bleached in water with sodium hypochlorite for 24hours after which the remaining soft tissues was teased-off. The intact bone was then cleansed in water, sun-dried and observed for any morphological alterations.

Samples of mandibular lymph node and salivary glands were also collected and fixed in Bouin's fluid for 72hours, dehydrated in graded alcohol, cleared in two jars of xylene and embedded in paraffin wax. Sections of 5µ thick were stained with Haematoxylin and Eosin (H & E). All the slides were examined under the Olympus BX 50 light microscope at x400 magnification to evaluate the histopathological features.

Results

Gross examination of the mandible showed a fistulous tract (4mm in diameter), with uneven ridges around its edges, on the lateral aspect, 4cm rostral to the angle of the ramus of the right mandible, extending inwards and ventral to the 2nd molar (Fig. 1). Infiltration of water from mandibular foramen reveals patency at the right molar teeth (M1 - M3) implying that the fistulous tract makes communication with the mandibular canal.

The alveolar borders of both sides of the mandible were worn off with the right side forming a thicker ridge than the left (Fig.2). There was the presence of foreign material (tough grasses) in the alveoli which remained stuck to the alveoli of the right side even after the hot water maceration of the mandible (Fig. 1). The mandibular tuberosity on the median surface of the right mandible was observed to be more prominent than the left mandible. Also, the alveolar alignment was distorted with deviation towards the median plane(Fig. 3).

Histological slides of the right mandibular lymph node revealed moderate fibroplasia, lymphoid hypoplasia with localized area of mineralization and mononuclear cells infiltration (mostly macrophages) (Fig 4).

Discussion

Reports on mandibular osteomyelitis in small ruminants are rare in the literature (Smith and Sherman, 1994). This report revealed the abnormal morphological changes due to *Actinomycesviscosus* infection in WAD goat. The observed fistulous tract with uneven ridges around its edges on the lateral horizontal aspect of the ramus of the right mandible, extending inwards ventral to the right molar was similar to that which was reported in the Sannen goat caused by *Arcanobacteriumpyogenes* (Seifi *et al.,* 2003) and in the sheep caused by Pseudomonas aeruginosa (Benito-Pena *et al.,* 2010). This shows perhaps, that the mandibular morphological changes due to Actinomycosis are similar in small ruminants even when the causative agents differ. According to Jack (1982), these changes are often sequel to the osteomyelitis and osteolysis that results from the acute osteitis of the mandible.

According to Thompson (2007), the normal bacterial flora of the oral cavity such as Actinomycesspp, can produce mandibular osteomyelitisin domestic sheep that present with oral trauma resulting fromthe ingestion of rough feed. Although the pathway of the infection in this case was not known, the natural response of the animal to pain (grinding of the teeth) and the presence of foreign material (tough grasses) in the alveoli coupled with the wearing off of the alveolar boarders and alignment suggest that there was trauma to the buccal mucosa which became infected. This also agrees with the finding that mandibular *osteomyelitis* occurs secondary to periodontitis (Seifi *et al.,* 2003).

In sheep, Benito-Pena *et al.* (2010) observed that the sub-mandibular and retropharyngeal lymph nodes were enlargedand congested with severe oedema. However in this case,moderate cortical fibroplasia with slight mononuclear phagocytes in the sinuses of the sub-mandibular lymph node was observed. It means though the lymph node was affected,perhapsthrough the gingival lymphatics that drain the alveolar bone (Jubb *et. al.,* 1985), healing process was in progress. According to Walker (1999), treatment of mandibular osteomyelitis in animals other than cattle is generally unsuccessful except when instituted early and aggressively with the right antibacterial agents. In this case the

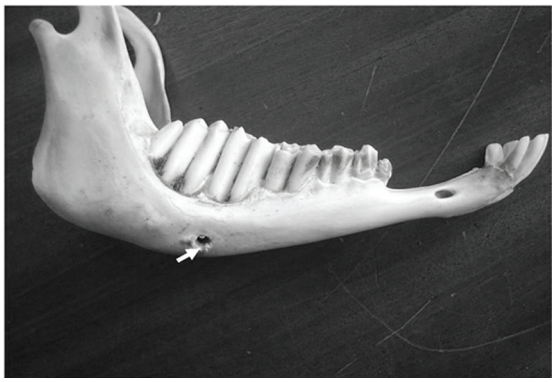

Figure 1: Photograph of the lateral view of the right mandible showing the fistulous tract and uneven ridges at its edge (arrow).

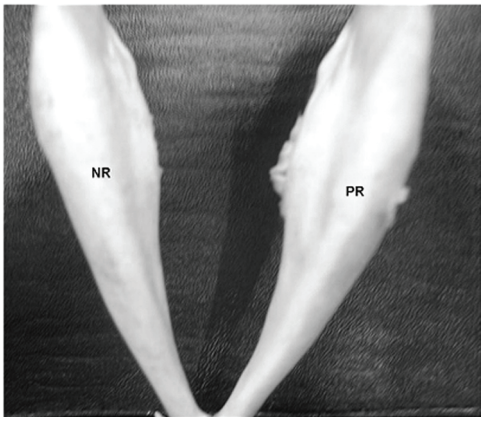

Figure 2: Ventral view of the mandible showing prominent mandibular ridge (PR) on the affected right mandible and the normal left mandibular ridge (NR).

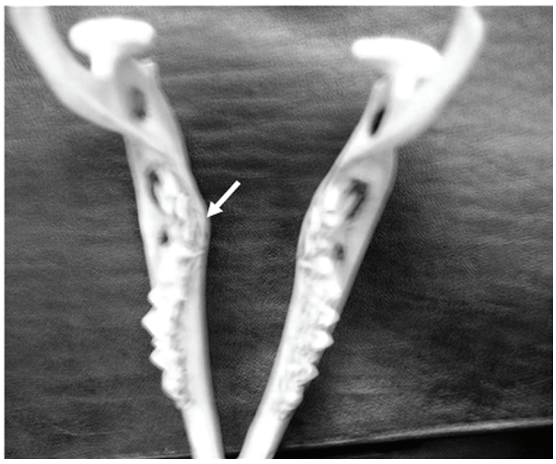

Figure 3: Dorsal view of the mandible showing medial deviation of the right mandible and the prominent ridge (arrow).

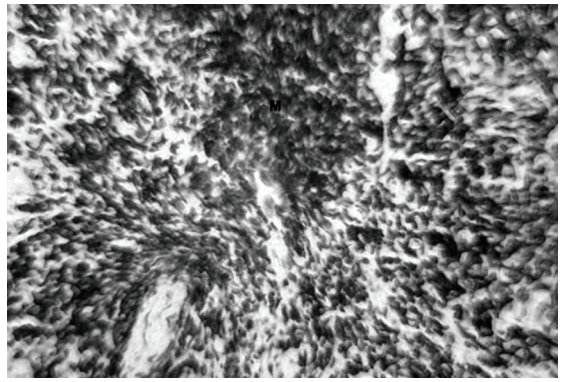

Figure 4: Photomicrograph of the mandibular lymph node showing the mild localized mineralized area (M) and mild diffuse area of fibroplasia. H& E X 400.

goat recovered from the infection but the morphological alterations of the mandible did not show any sign of recovery.

In conclusion it seems that thepathological processes that led to these alterations are similar in small ruminants irrespective of the causative agents and can result to permanent imbalance in the alignment of the upper and the lower jaws thereby impairing chewing and consequently, the growth of the affected and even treated animals.

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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
December 2011

Preamble
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.

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- Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- 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.
- Reports: Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- 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.
- Web links: Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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