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IMPACT ECONOMIQUE DE L'EPIZOOTIE DE PESTE EQUINE DE 2007 AU SENEGAL

ECONOMIC IMPACT OF AFRICAN HORSE SICKNESS OUTBREAK IN SENEGAL IN 2007

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Résumé

La présente étude a été menée afin d'estimer l'impact économique de cette épizootie au Sénégal en vue de fournir aux décideurs des arguments pour une stratégie de lutte pluriannuelle et durable. L'approche méthodologique adoptée est basée sur l'utilisation des données secondaires disponibles au niveau du service public et du service privé. Les calculs électroniques et manuels ont été utilisés pour le traitement des données et une analyse coûts-avantages a été faite pour comparer les avantages liés à la lutte par rapport au coût de la lutte. L'étude révèle que l'épizootie a entraîné un total de 1169 morts sur un effectif national de 518212 chevaux estimés et un total de 1357 malades sur un effectif de 517614 chevaux traditionnels estimés. Le coût économique total a été estimé à 896790798 FCFA. Les pertes liées à la mortalité et à la morbidité représentent la part la plus importante de ce coût avec 498462665 FCFA, soit 55,58 % du coût économique total. Le reste du coût économique total (398328133 FCFA, soit 44,42 %) est dû au coût de contrôle de la maladie. L'investissement fait au début de l'épizootie a produit un résultat positif avec un ratio coûts-avantages de 1,25. Il ressort de cette étude qu'il est donc possible, à travers une stratégie de prévention bien menée, par la vaccination et la lutte contre les vecteurs par exemple, de limiter de façon durable l'impact économique de la peste équine au Sénégal.

Mots clés: Impact économique – Peste équine – Analyse coûts-avantages –Contrôle de la maladie – Sénégal

Abstract

This study aimed at estimating the economic impact of the disease in Senegal in order to provide to policy makers arguments for a sustainable multi-year control strategy. The methodological approach adopted was based on the use of available secondary data in the public and private animal health services. A survey on local livestock markets has been made to better estimate the values of horses and thus better quantify the economic losses associated with the disease. Electronic and manual calculations were used for data processing and cost-benefit analysis conducted to compare the benefits from the control over the costs involved.

This study shows that the disease caused a total of 1169 deaths out of a national herd of about 518212 horses, i.e a national mortality rate of 0.23%. The outbreak caused a total of 1357 sick animals out of 517614 horses, i.e. a national morbidity rate of 0.26% in the traditional farming systems. The total economic cost of the 2007 outbreak of African horse sickness in Senegal was estimated at 896790798 FCFA. Losses related to mortality and morbidity represent the largest share of that cost with 498462665 CFA francs, or 55.58% of total economic costs. The remainder of the total economic cost (398328133 CFA francs, or 44.42%) is due to the cost of controlling the disease. The investment made at the beginning of the outbreak has produced a positive result with a cost-benefit ratio of 1.25. If the authorities had invested 398328133 CFA francs for the prevention against horse sickness, a loss of 498462665 FCFA would have been avoided. Therefore it is possible, through a strategy of prevention carried out by immunization and vector control to limit in a

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sustainable manner the economic impact of African horse sickness in Senegal.

Keywords: Economic Impact - African horse sickness - Cost-benefit analysis - control of the disease - loss of production – Senegal.

Introduction

La peste équine est une maladie redoutable de l'espèce équine. Elle entraîne de lourdes pertes dans les zones nouvellement infectées avec parfois 100% de mortalité. Elle constitue de ce fait l'un des risques sanitaires majeurs des équidés. Au Sénégal, l'épizootie de 2007 a replacé le cheval au centre de l'actualité nationale, remettant ainsi son importance au premier rang des préoccupations des autorités sanitaires du pays. L'élevage du cheval revêt un intérêt socio-économique certain. En effet, le cheval est présent dans plusieurs activités comme : le transport des hommes et des marchandises, les courses hippiques et l'équitation de loisir en milieu urbain ; le transport, les travaux champêtres en milieu rural. Il garde son rôle social dans la communauté et dans les cours royales ou de l'ancienne noblesse de certains pays, et figure en bonne place dans le développement de nombreux métiers liés au cheval. Du fait de cette grande importance, le cheval devrait faire l'objet d'une attention particulière, ne serait ce que pour amoindrir les contraintes liées à son élevage, particulièrement les contraintes sanitaires qui occupent une place majeure. Les pertes économiques engendrées aux propriétaires de chevaux et à l'Etat par la maladie, font que des programmes de contrôle s'imposent. Pour une stratégie de lutte durable, il faut que les gains économiques induits soient établis en plus de la maîtrise de la maladie. Dans les pays en développement, les financements sont rares et difficiles à obtenir. Aussi, tout plan de lutte de grande envergure doit être précédé d'une évaluation de la rentabilité économique de l'opération afin de fournir aux décideurs les informations nécessaires pour la prise de décision.

La présente étude vise à estimer l'impact économique de l'épizootie de peste équine survenue au Sénégal en 2007. Spécifiquement, la détermination du coût ou valeur marchande des équidés selon leur utilisation, permet de mieux quantifier les pertes économiques liées à la peste équine et de juger de la rentabilité de la lutte, au regard du coût de la lutte.

Matériel et méthodes

Méthode de collecte des données

Collecte des données pour évaluer la valeur marchande des chevaux

Au Sénégal, il n'existe pas de statistiques fiables sur les prix des chevaux. Cette situation a justifié le recours à une enquête pour avoir une idée du prix moyen des chevaux. Deux catégories de chevaux ont été identifiées : les chevaux de race améliorée (chevaux de courses, reproducteurs et d'équitation) et les chevaux traditionnels ou de race locale. Les prix des chevaux traditionnels ont été obtenus par le biais d'une enquête au niveau des marchés hebdomadaires des localités de Touba-Toul et de Dahra du fait de leur importance dans le commerce des chevaux au Sénégal. Un échantillon de 12 marchands a ainsi été enquêté dont 4 au marché de Touba-Toul et 8 au marché de Dahra. Les marchands ont été choisis en fonction de leur disponibilité à répondre au questionnaire. L'administration du questionnaire s'est faite sous forme d'entretien en langue locale (Wolof). Les chevaux de race améliorée ne sont pas commercialisés sur les marchés de Dahra et Touba-Toul. Leur valeur a été obtenue auprès de leurs propriétaires par entretien direct.

Collecte des données épidémiologiques

Le nombre de cas clinique, les mortalités ainsi que l'évolution de la maladie dans le temps ont constitué les données épidémiologiques. Elles ont été obtenues au

niveau de la Direction de l'Elevage (DIREL). A ce niveau, les fiches d'enregistrement journalier des mortalités de tout le pays transmises par les inspecteurs régionaux (IRSV) et départementaux des services vétérinaires (IDSV), ont été dépouillées et ont constitué notre base de données de travail. Ces informations peuvent aussi être transmises par les chefs de postes vétérinaires et les techniciens d'élevage, qui sont de véritables collecteurs de données. Les moyens de communication sont : le téléphone, le système Intranet de la DIREL, etc.

Collecte des données sur le diagnostic

Les données sur le nombre de sérums envoyés au laboratoire à des fins de diagnostic ont été obtenues au niveau du service de virologie du Laboratoire National d'Elevage et de Recherches Vétérinaires (LNERV) de Dakar. Les prix des analyses ainsi que le coût d'envoi des échantillons d'organes pour analyse au laboratoire de Pirbright au Royaume Uni et au Onderstepoort Veterinary Institute en Afrique du Sud ont été obtenus dans les services administratifs du LNERV.

Collecte des données sur la vaccination

Les données sur le nombre de doses de vaccins utilisées par les services publics ont été obtenues à partir des rapports de vaccination de la DIREL ; et celles utilisés par les vétérinaires privés ont été fournies par les inspecteurs régionaux des services vétérinaires. Les prix des vaccins monoéquipeste et polyéquipeste ont été fournis par le service de vente du LNERV.

Collecte des données sur la lutte contre les vecteurs

Les données sur le nombre d'élevages désinfectés ont été obtenues au niveau des Inspections Départementales des Services Vétérinaires (IDSV) et des services d'hygiène de Rufisque et de Pikine. Les produits utilisés pour la désinfection sont : le Virkon®, le Dursban®, le Propoxur® 2 %, l'eau de javel et le Crésyl®. Les prix de ces produits ont été obtenus dans les services des IDSV de Rufisque et de Pikine et des services d'hygiène des mêmes départements.

Collecte des données de la coordination

Les données sur la coordination des différentes actions de lutte portent sur l'achat du petit matériel et outillages techniques (seringues, aiguilles, alcool, coton, glace) et sur la communication/sensibilisation (achat de tee-shirts, de casquettes, confection de banderoles, de dépliants, et pour des spots à la télévision et à la radio). A ces données, il faut ajouter les celles sur les frais de mission de supervision et des réunions, les frais du carburant nécessaire pour les déplacements des agents de l'Etat pour la vaccination, la supervision et les données sur le nombre et les prix des véhicules et matériel utilisés dans la lutte qui ont été obtenues à la DIREL. Le nombre et les prix du matériel employé dans la lutte (véhicules, matériel de froid, etc.) ont été obtenus au Ministère de l'Elevage.

Méthodes de traitement des données

Les données collectées sur les marchés ont fait l'objet d'un simple traitement manuel. Les données obtenues auprès des services publics et techniques, ainsi qu'au niveau des laboratoires ont fait l'objet d'une saisie et d'un traitement par calcul électronique. La méthode de traitement pour chaque rubrique est présentée ci-dessous.

Pertes dues à la mortalité
Calcul du taux de mortalité

Le taux de mortalité a été calculé en tenant compte de la typologie des élevages des chevaux en élevages traditionnels et modernes. Dans les élevages traditionnels, le taux de mortalité (rapport du nombre de mortalités dans la région concernée au nombre total de chevaux traditionnels estimés dans la région multiplié par 100) a été calculé par région. Le taux de mortalité

au niveau national dans les élevages traditionnels est le rapport du nombre total de mortalités sur le plan national au nombre total de chevaux traditionnels estimés au Sénégal multiplié par 100.

Dans les élevages modernes, le taux de mortalité au niveau régional (nombre total estimé de chevaux modernes dans la région) a été calculé. Sur le plan national, le taux de mortalité dans les élevages modernes est le rapport du nombre total de mortalités national dans les élevages modernes au nombre total de chevaux modernes possédant un certificat d'origine. Ces taux de mortalités partiels ont ensuite fait l'objet d'un cumul pour obtenir le taux global de mortalité.

Estimation des pertes par mortalité

Les pertes dues à la mortalité sont exprimées en termes de coût de remplacement du cheval mort. Dans les élevages modernes, elles sont obtenues en faisant le produit du nombre des équidés morts par race par le prix moyen du cheval mort correspondant. Les prix moyens utilisés sont ceux obtenus directement auprès des responsables des écuries de la zone périurbaine de Dakar.

Dans les élevages traditionnels, la perte est obtenue en faisant le produit du nombre des équidés morts par classe d'âge par le prix moyen du cheval traditionnel de remplacement. Les prix des chevaux utilisés sont des prix moyens obtenus sur les marchés de Touba-Toul et Dahra après enquêtes. L'enregistrement des mortalités a été fait sans tenir compte des différentes classes d'animaux. Or cette information est nécessaire pour une évaluation correcte de la valeur des mortalités. La structure des mortalités selon l'âge et le sexe a été obtenue suivant des estimations en pourcentage, faites par des Inspecteurs Régionaux des Services Vétérinaires. Pour ce faire un tableau à remplir a été soumis à ces derniers. Pour la région de Kaolack par exemple, l'effectif total des mortalités est représenté par KL. Les adultes (A) représentent KL * 87 % ; et les jeunes : KL *13 %. Parmi les adultes (A), les étalons représentent A * 32 % et les juments A * 68 %. Le même procédé de calcul est utilisé pour les autres régions (tableau I).

(* = multiplié par)

Pertes dues à la morbidité

Calcul du taux de morbidité

Le taux de morbidité est le rapport du nombre de chevaux malades rapporté à l'effectif national estimé, multiplié par 100.

Estimation des pertes par morbidité

La réduction, voire la perte de la

Tableau I: Structure des mortalités par classe d'âge et sexe

Région	Total mortalité	Adultes (A)	Etalons	Juments	Jeunes
Kaolack	KL	KL * 87 %	A * 32 %	A * 68 %	KL * 13 %
Tambacounda	TB	TB * 83 %	A * 60 %	A * 40 %	TB * 17 %
Louga	LG	LG * 85 %	A * 22 %	A * 78 %	LG * 15 %
Diourbel	DL	DL * 100 %	A * 86 %	A * 14 %	DL * 0 %
Fatick	FK	FK * 75 %	A * 62 %	A * 38	FK * 25 %
Thiès	TH	TH * 87 %	A * 87 %	A * 13 %	TH * 13 %
Matam	MT	MT * 77 %	A * 25 %	A * 75 %	MT * 23 %
St Louis	SL	SL * 80 %	A * 24 %	A * 76 %	SL * 20 %
Dakar	DK	DK * 70 %	A * 40 %	A * 60 %	DK * 30 %

force du travail du cheval malade ou mort a été considérée comme une perte due à la morbidité. En effet, la force de travail d'un cheval malade diminue durant la période allant de la fin de l'incubation jusqu'à la guérison complète alors que cette force de travail est perdue pour le cheval mort. En considérant que les chevaux morts ont d'abord été malades, le nombre total de chevaux malades, a inclus ceux qui ont présenté les symptômes et qui ont guéri en plus de ceux qui sont morts. Les pertes dues à la morbidité ont été obtenues en multipliant le nombre de chevaux malades par la durée moyenne de la maladie et par le coût d'opportunité journalier d'un cheval sain, apte à travailler.

Les informations recueillies auprès des différents Inspecteurs régionaux des services vétérinaires permettent d'affirmer que la maladie a eu en général une évolution aiguë, conduisant le plus souvent à la mort au cours des 24 à 72 h après l'observation des symptômes. Compte tenu de ce résultat, une durée moyenne de 48 h soit 2 jours est retenue dans le calcul de la morbidité.

En raison d'un manque d'information sur le coût d'opportunité journalier de l'utilisation des chevaux traditionnels, les résultats obtenus par LY et coll. (1998) dans la ville de Thiès ont été utilisés. En effet, ces auteurs ont obtenu des marges nettes journalières respectives de 2160 et 2737 FCFA par les fiacres et les charrettes. La valeur moyenne de ces marges nettes qui est de 2500 FCFA va être utilisée dans cette étude comme étant le coût d'opportunité journalier de l'utilisation d'un cheval traditionnel en bonne santé.

Pour les chevaux issus des élevages modernes, en dehors des jours de compétition, ils ne sont pas soumis à un quelconque travail quotidien rémunérateur. A cet effet, il est difficile de quantifier cette perte car la valeur des chevaux morts inclut généralement le coût de la morbidité parce qu'elle tient compte de plusieurs paramètres dont le degré de pureté raciale, gain aux compétitions, etc.

Coût de contrôle de la maladie

Le contrôle de la maladie a été obtenu par le diagnostic des sérotypes circulant dans le pays, une campagne de vaccination de masse et la lutte contre les vecteurs dans les écuries modernes. Les traitements médicaux n'ont pas été pertinents pour être pris en compte dans ce document. Le contrôle de la maladie a nécessité des fonds de l'Etat sénégalais mais aussi un ensemble de mesures administratives et sanitaires visant à diagnostiquer la maladie et les sérotypes, à vacciner les chevaux et enfin à lutter contre les vecteurs autour et dans les écuries modernes.

Le coût du diagnostic

Le coût du diagnostic est la valeur de l'ensemble des moyens mis en œuvre pour le sérotypage du virus. Il est représenté par le coût de l'analyse des échantillons de sérums effectués au LNERV, mais aussi les frais d'envoi des échantillons d'organes pour analyse au laboratoire de Pirbright au Royaume Uni et à l'Onderstepoort Veterinary Institute en Afrique du Sud. Les sérums envoyés au LNERV pour analyse ont été soumis systématiquement à la recherche du sérotype 2 et du sérotype 9. Le coût de l'analyse des sérums est obtenu en faisant le produit du nombre de sérums analysés par le prix du sérotypage. Les échantillons envoyés pour analyse au Royaume Uni et en Afrique du sud sont constitués de fragments d'organes de chevaux morts qui ont été analysés gratuitement. Les envois sont faits par colis de 1 kg contenant les échantillons d'organes et pour certains colis, des échantillons de vaccins en plus des organes. Les envois ont été faits en Mars, Mai et Août, périodes correspondant au début de chaque phase de l'épizootie. Le coût d'envoi est obtenu en faisant le produit du nombre d'envois par le prix unitaire de l'envoi, prix proposés par la compagnie SAGA SENEGAL.

Le coût de la vaccination

Le coût de la vaccination est

représenté par la valeur de l'ensemble des moyens mis en œuvre pour vacciner les chevaux. Il est obtenu par le produit du nombre de chevaux vaccinés en utilisant les vaccins polyéquipeste ou monoéquipeste par le prix du vaccin auquel on ajoute le coût de l'administration du vaccin effectuée par les vétérinaires privés. Au début de la campagne de vaccination d'urgence en effet, il y a eu une courte période, avant la décision de l'Etat sénégalais de prendre en charge totalement la vaccination. Durant cette période, certains vétérinaires privés ont vacciné et se sont fait rémunérer selon des vaccinateurs entre 1100 FCFA et 2100 FCFA par cheval. Une moyenne de 1600 FCFA a été utilisée pour calculer ces coûts supplémentaires payés par les éleveurs. Les échantillons de vaccins envoyés au laboratoire de Pirbright, pour contrôle de qualité, ont été inclus dans les mêmes colis que ceux des échantillons d'organes destinés au même laboratoire. Les frais d'envoi des échantillons de vaccins ont par conséquent été pris en compte dans le coût du diagnostic.

Coût de la lutte contre les vecteurs

Le coût de la lutte contre les vecteurs est représenté par la valeur de l'ensemble des moyens mis en œuvre pour la désinfection des écuries modernes. Il est constitué du coût des désinfectants et du coût du carburant qui a été utilisé pour effectuer le déplacement dans les élevages concernés. Le coût des désinfectants est obtenu en faisant le produit de la quantité du produit utilisé par le prix unitaire du produit considéré. Le coût du carburant est le produit de la quantité estimée du carburant utilisé par son prix unitaire. Le coût des désinfectants tels que l'eau de javel et le crésyl a été fourni par les IDSV. Les prix des produits utilisés sont des prix de Novembre 2007. L'amortissement du matériel et du véhicule utilisés pour la désinfection n'a pas été pris en compte du fait de leur utilisation ponctuelle.

Coût de la coordination

Le coût de la coordination est représenté par la valeur des moyens mis en œuvre pour gérer la lutte qui a permis d'arrêter la maladie. Les coûts d'achat du petit matériel et outillages techniques, les frais de mission de supervision et des réunions, les coûts de la communication/ sensibilisation et les frais du carburant ont été utilisés tels qu'ils ont été fournis par la DIREL.

L'amortissement pour chaque véhicule et matériel a été considéré comme linéaire et correspondant au rapport entre la valeur du matériel à l'achat sur sa durée probable d'utilisation. Les valeurs résiduelles ont été considérées comme nulles puisque le contrôle de l'épizootie de 2007 de la peste équine a fait appel à une gamme de matériel d'âges variés. La valeur des véhicules et matériel amortis utilisés dans la lutte n'a pas été prise en compte. Les prix des différents matériels non amortis ont été uniformisés afin de faciliter les calculs. Ces prix qui étaient en vigueur en 2007, sont exprimés en FCFA toutes taxes comprises.

Du fait de la diversité du matériel de froid utilisé, le prix de ce matériel a fait l'objet d'une standardisation selon la méthodologie de LY et coll. (1998) dans le cadre d'une analyse des coûts d'une campagne officielle de prophylaxie animale. En effet, les réfrigérateurs sont assimilés à des réfrigérateurs de 320 litres et d'une puissance de 195 watts et les congélateurs à ceux de 350 litres et d'une puissance de 200 watts. Les glacières sont assimilées aux glacières de 15 litres. Les frais de consommation électrique du matériel de froid ont été estimés à partir de leur puissance et de leur durée d'utilisation par jour, évaluée à 24 h en fonction de la tarification de la société nationale d'électricité du Sénégal qui s'élevait en moyenne à 102 F CFA/KWh. Les frais des communications téléphonique n'ont pas été pris en compte du fait d'un manque d'information. Tous les calculs ont été effectués pour une durée de lutte de cinq mois (Août à Décembre 2007).

Estimation de l'impact économique de la peste équine et analyse coûts-avantages

Impact économique de la peste équine

L'estimation de l'impact économique de la peste équine a été faite en termes de coût économique. Celui-ci se traduit par la relation entre la valeur des pertes évitées grâce à la lutte et le coût de contrôle de la maladie. Les pertes économiques totales (C) sont obtenues en additionnant les valeurs des pertes directes et indirectes de production (L) résultant de la mortalité et de la morbidité, additionnées du coût de contrôle (E) soit : C = L + E

Les pertes directes de production considérées ont concerné la diminution du nombre d'équidés (mortalité) et la perte de la force de travail (morbidité) alors que les coûts de contrôle considérés ont concerné le coût de la vaccination, ceux du diagnostic, de la lutte contre les vecteurs et du coût de la coordination de la lutte. Les pertes indirectes résultant de la diminution de la fertilité et le retard sur la mise en vente des chevaux n'ont pas été prises en compte du fait de la non disponibilité de données.

Analyse coûts-avantages

L'analyse coûts-avantages prend en compte la variation des gains et des coûts entre deux situations, l'une « avec » et l'autre « sans » programme de lutte. Concernant les gains, deux types sont à considérer à savoir les gains directs et les gains indirects.

Les gains directs ont été estimés à partir des pertes évitées imputables à la mortalité et à la morbidité ou de l'épargne du coût de contrôle. En effet, un contrôle approprié avec la vaccination après identification du sérotype ou la lutte contre les vecteurs, élimine ou réduit les pertes dues à la mortalité et à la morbidité. L'animal ayant survécu est considéré comme un gain et sa valeur est mesurée en terme de coût de remplacement. Un cheval atteint de peste équine, perd sa valeur et connaît une diminution de ses potentialités de production en terme de force de traction, aptitudes physiques à la course, diminution de la fertilité et enfin la diminution des produits dérivés (lait, viande, produits d'origine biologiques, etc.). L'élimination de la maladie permet à l'animal d'exprimer toutes ses potentialités de production. L'éradication de la peste équine permet d'éviter tous les coûts de contrôle futur (diagnostic, vaccination, lutte contre les vecteurs, quarantaine, surveillance et contrôle de mouvements), fournissant ainsi un bénéfice aux détenteurs de chevaux et à l'Etat.

Les gains indirects surviennent lorsque le contrôle ou l'éradication de la peste équine, ouvre à nouveau le marché avec les pays ou des régions avec lesquels il n'était plus possible d'échanger les animaux à cause de la maladie. La fermeture d'un pays au marché international constitue, en effet, une perte à la fois pour les vendeurs et pour les acheteurs de chevaux mais aussi pour l'Etat qui n'enregistre plus de rentrées de taxes ni de devises. Cependant, le coût de la mise en œuvre du contrôle des mouvements des animaux et des procédures de la quarantaine est significatif et cela sous-évalue les gains, lorsqu'ils ne sont pas pris en compte lors du calcul de l'impact économique.

L'analyse coûts-avantages a été limitée seulement aux gains directs survenant par l'économie des coûts de contrôle et les pertes évitées dues à la mortalité et à la morbidité. Cela suppose un programme « avec » et « sans » contrôle de la maladie en considérant que les mortalités sont enregistrées lorsque aucun plan de lutte n'est mis en place. Les coûts et les gains ont été mesurés en terme de variation de leurs valeurs additionnelles entre le programme « avec » et « sans » contrôle de la maladie. Le coût additionnel a été considéré comme étant la différence entre les dépenses supplémentaires des programmes « avec contrôle » et « sans contrôle ». Le gain additionnel a par contre été considéré comme étant la différence entre la valeur de production (pertes évitées) obtenue

avec le programme de contrôle et la valeur obtenue sans le contrôle (pertes).

L'analyse coûts-avantages a été utilisée pour comparer la valeur du gain additionnel avec la valeur du coût additionnel pour établir si le contrôle est économiquement rentable ou non selon la méthode utilisée par TAMBI et coll. (2006)

$RCA = [\sum At / (1 + r)t] / [\sum Ct / (1 + r)t]$,
où : RCA = ratio coûts-avantages ;
A = le gain à échéance du programme de contrôle ;
C = le coût de contrôle de la maladie ;
r = le taux d'escompte ; et t = nombre d'années dans le futur.

Un ratio coûts-avantages supérieur à 1 signifie que le contrôle de la peste équine est économiquement viable tandis qu'un ratio inférieur à 1 signifie le contraire.

Résultats et discussions

Enquêtes sur les marchés de chevaux
Caractérisation du marché des chevaux
Flux Physique

Parmi les 12 marchands de chevaux enquêtés, 11 (92 %) affirment que les chevaux se vendent plus facilement à l'approche de la saison chaude et humide (hivernage) c'est-à-dire durant la période allant d'avril à juillet. Au cours de cette période, les 12 marchands affirment que les prix augmentent mensuellement pour atteindre leur maximum en juillet-août et rechutent à partir de la période post-hivernale c'est-à-dire du mois de novembre jusqu'en mars. Le nombre de chevaux vendus par mois varie donc en fonction de la période qui peut aller d'un minimum de 1 cheval à un maximum de 22 chevaux pour certains marchands. L'enquête révèle que les races de chevaux vendus sont principalement les races locales.

Flux monétaires

Les prix des chevaux locaux varient d'un minimum de 70 000 FCFA à un maximum de 250 000 FCFA chez les jeunes âgés de moins de 4 ans, d'un minimum de 150 000 FCFA à un maximum de 350 000 FCFA chez les juments et d'un minimum de 200 000 FCFA à un maximum de 400 000 FCFA chez les étalons (tableau II). Tous les marchands enquêtés affirment que ces prix sont variables en fonction de la période de l'année, du sexe, de l'âge, de l'état d'embonpoint et de l'état sanitaire des chevaux. Cette variation croissante des prix des chevaux à l'approche et pendant l'hivernage s'explique par le fait que c'est à cette période que les chevaux sont les plus sollicités pour les travaux champêtres et leurs demandes est de ce fait forte sur les marchés. La conséquence de cette augmentation de la demande, en supposant fixe l'offre du marché, est l'augmentation des prix selon la loi de l'offre et de la demande. (FAYE, 1988)

Devenir des chevaux achetés

Selon les marchands interrogés, 11 chevaux achetés sur 12 sont utilisés dans la traction (transport, travaux champêtres, etc.) et seulement 1 cheval sur 12 est utilisé pour la reproduction.

Tableau 2: Prix des chevaux en fonction de l'âge et sexe

Catégories	Minimum (FCFA)	Prix maximum (FCFA)	Moyen (FCFA)
Jeunes	70 000	250 000	160 000
Juments	150 000	350 000	250 000
Etalons	200 000	400 000	300 000

Effets de la peste équine sur le commerce des chevaux

L'enquête révèle que tous les marchands connaissent la peste équine et trois parmi eux ont enregistré des cas de mortalités. Les animaux morts étaient tous des adultes de plus de cinq ans.

Effets négatifs

Sur 12 marchands interrogés, 11 affirment que l'épizootie de 2007 de la peste

équine a constitué un frein à leur commerce car ayant provoqué la diminution du nombre de chevaux vendus à cause de la réticence des clients qui ne sont plus certains de l'état sanitaire des animaux vendus.

Effets positifs

Parmi les 12 marchands enquêtés, un seul a affirmé que la maladie a constitué un atout à son commerce car les utilisateurs qui ont perdu leurs chevaux venaient sur le marché rechercher des chevaux de remplacement.

Pertes par mortalité et par morbidité
Pertes par mortalité
Taux de mortalité

L'épizootie a entraîné un total de 1137 morts sur un effectif estimé de 517 614 chevaux dans les élevages traditionnels, soit un taux de mortalité au niveau national de 0,22 % et 32 morts dans les élevages modernes sur un effectif estimé de 598 chevaux soit un taux de mortalité au niveau national de 5,35 %.

Les taux de mortalité dans les élevages traditionnels varient de 0,04 % dans la région de Diourbel à 0,56 % dans la région de Saint-Louis. Il n'a pas été enregistré de mortalités dans les régions du sud du pays (Ziguinchor et Kolda). Par contre, les régions de Kaolack, Fatick, Louga et Thiès sont des régions ayant enregistré le plus de mortalités avec plus de 78 % de cas enregistrée sur les mortalités totales. La maladie a entraîné une perte totale de 536 juments, 418 étalons et 183 jeunes (poulains et pouliches).

Dans les élevages modernes, l'essentiel des mortalités a été enregistré à Dakar avec 30 mortalités soit 8,57 % au niveau régional et 2 mortalités soit 10 %dans la région de Diourbel.

Le cumul des mortalités survenues à la fois dans les élevages traditionnels et dans les élevages modernes au niveau régional et national permet de se rendre compte que la région de Dakar, avec un cumul de 0,48 %, présente un taux de mortalité proche de celui observé dans les régions de St Louis et de Kaolack (tableau III).

Il ressort de l'étude que contrairement aux précédentes années, l'épizootie de 2007 a eu une incidence plus importante au niveau national, et est qualifiée par certains experts, comme étant la plus meurtrière de l'histoire du pays. Ceci est à relier à l'apparition d'un nouveau sérotype : le sérotype 2 contre lequel aucune vaccination n'était effectuée.

Ces résultats montrent également que le taux de mortalité est plus faible dans les élevages traditionnels (0,22 %) que dans les élevages modernes (5,35 %). Cela pourrait s'expliquer en reprenant les arguments de BOURDIN et LAURENT (1974) selon lesquelles les animaux importés ou qui ont subi une amélioration de leur matériel génétique sont moins résistants aux pathologies. En effet, les chevaux de race améliorée élevés au Sénégal proviennent pour la plupart des zones indemnes de peste équine d'Europe, contrairement aux chevaux traditionnels qui sont en contact permanent avec le virus et qui ont développé avec le temps une immunité contre la maladie.

Les taux de mortalité enregistrés sont faibles (0,22 % et 0,26 %) par rapport à ce que rapportent LEFEVRE et coll. (2003) (70 à 90) Ces résultats pourraient s'expliquer par le fait que le premier foyer a été observé en février-mars, période défavorable à la multiplication des vecteurs (restriction de la diffusion) et dans des écuries modernes où les animaux coûtent très chers. Cette situation a conduit les autorités sénégalaises à s'activer pour contenir la maladie. Une grande mobilisation du service public ainsi que des moyens matériels et financiers a été observée. Il y a eu un renforcement de l'appui de l'Etat au niveau opérationnel par la mise à la disposition de certains agents de terrain de nouveaux véhicules, de motos, et l'affectation de certains matériels destinés à la surveillance d'autres maladies. En outre, une bonne campagne médiatique a été menée, ce qui a contribué, avec la vaccination gratuite des chevaux, à un

Tableau 3: Mortalité et taux de mortalité cumulé dans les élevages modernes et traditionnels

Région	Départements	Nombre de morts	Total de morts régional	Pourcentage des mortalités	Effectif régional	Taux de mortalité cumulé
Kaolack	Kaolack	177	453	38,75	117 900	0,38
	Nioro	17				
	Kaffrine	259				
	Tambacounda	65				
Tambacounda	Bakel	6	71	6,07	31 800	0,22
	Kédougou	0				
	Linguère	108				
Louga	Kébémer	13	136	11,63	62 500	0,22
	Louga	15				
	Bambey	19				
	Mbacké	1				
Diourbel	Diourbel	11	31	2,62	70 570	0,04
	Gossas	56				
	Foundioungne	35				
Fatick	Fatick	80	171	14,63	85 000	0,20
	Mbour	51				
	Thiès	16				
Thiès	Tivaouane	62	129	11,04	59 100	0,22
	Ranérou	25				
	Matam	24				
Matam	Kanel	29	78	6,67	26 600	0,29
	Dagana	23				
	Podor	42				
Saint-Louis	Saint-Louis	2	67	5,73	11 900	0,56
	Rufisque	16				
	Dakar	1				
	Pikine	16				
Dakar	Guédiawaye	0	33	2,82	6 910	0,48
	Kolda	0				0.00
	Vélingara	0		0.00		
Kolda	Sédhiou	0	0		42 500	
	Bignona	0				
	Ziguinchor	0				
Ziguinchor	Oussouye	0	0	0.00	3 204	0.00
Total national		**1169**	**1169**	**100,00**	**518 212**	**0,23**

diagnostic précoce du sérotype circulant, à la désinfection de certains haras privés et au confinement des animaux, à la réduction de la diffusion de la maladie.

Coûts des pertes par mortalité

Le coût lié à la mortalité dans les élevages traditionnels s'élève à 288 655 165 FCFA (tableau IV) et celui des élevages modernes à 205 000 000 FCFA (tableau V) soit un coût total de la mortalité de 493 655 165 FCFA.

Coûts des pertes par morbidité

L'épizootie a entraîné un total de 1 357 malades sur un effectif de 517 614 chevaux soit un taux de morbidité national de 0,26 % dans les élevages traditionnels, ce qui se traduit par un coût en terme de perte de la force de travail de 4 807 500 FCFA.

Résultats de la lutte

Le coût du diagnostic

Au total, 886 sérums ont été analysés au LNERV et 17 échantillons d'organes envoyés à l'extérieur du Sénégal pour analyse. La majorité des sérums analysés (81,6 %) sont constitués par des prélèvements effectués à Dakar. Les envois ont été faits en 7 colis de 1 kg dont 6 à Pirbright et 1 en Afrique du sud. Le coût total du diagnostic a été estimé à 5 142 145 FCFA (tableau VII) dans lequel 4 252 800 FCFA soit 83 % représentent le coût total des analyses de sérums et 889 345 FCFA soit 17 % représentent le coût d'envoi des prélèvements à l'extérieur (tableau VI).

Le coût de la vaccination

Le nombre total de chevaux vaccinés avec le vaccin polyéquipeste s'élève à 175 300 têtes, soit un coût de vaccination avec le polyéquipeste de 245 420 000 FCFA. Les

Tableau 4 : Coûts des pertes par mortalité dans les élevages traditionnels (FCFA)

Région	Etalons	Juments Coûts	Jeunes	Total
Kaolack	37834560	66998700	9422400	114255660
Tambacounda	10607400	5893000	1931200	18431600
Louga	7629600	22542000	3264000	33435600
Diourbel	7482000	1015000	0	8497000
Fatick	23854500	12183750	6840000	42878250
Thiès	29292030	3647475	2683200	35622705
Matam	4504500	11261250	2870400	18636150
Saint-Louis	3859200	10184000	2144000	16187200
Dakar	252000	315000	144000	711000
Total	**125315790**	**134 040 175**	**29299200**	**288655165**

Tableau 5: Coût des pertes par mortalité dans les élevages modernes (FCFA)

Race	Nombre	Prix unitaire moyen	coût total
Pur sang	9	8000000	72000000
Améliorée	17	5000000	85000000
Etrangères (All, Belge,Selle français)	6	8000000	48000000
Total	**32**		**205000000**

All : Allemand

Tableau 6: Frais d'expédition des prélèvements

Destination	Nombre de colis	Coût unitaire (FCFA)	Coût total (FCFA)
Pirbrigth	6	121580	729480
Onderstepoort	1	159865	159865
Coût total			**889345**

Tableau 7: Estimation du coût du diagnostic

Rubrique	Coûts (F CFA)	Pourcentage(%)
Analyse des sérums	4252800	83
Envoie prélèvements	889345	17
Total	5142145	100

régions de Kaolack, de Louga et de Fatick occupent la moitié du coût de vaccination. La vaccination avec le vaccin monoéquipeste a concerné 5 896 chevaux à Dakar, soit un coût de vaccination de 5 306 400 FCFA. Un total de 35 099 chevaux a été vacciné par les vétérinaires privés avant que la décision de la gratuité des actes vaccinaux ne soit prise, soit un coût pour les éleveurs de 56 158 400 FCFA. Le coût total de la vaccination s'élève donc à 306 884 800 FCFA (tableau 8).

Le coût de la lutte contre les vecteurs

La lutte contre les vecteurs a concerné 10 écuries modernes dont 8 dans le département de Rufisque et 2 dans le département de Pikine. La lutte a coûté

Tableau 8: Coût total de la vaccination

Rubrique	Nombre vaccination	Prix unitaire (FCFA)	Coût total (FCFA)	Pourcentage
Vaccination avec Polyéquipeste	175300	1400	245420000	80,0
Vaccination avec Monoéquipeste	5896	900	5306400	1,7
Rémunération des vétérinaires privés	35099	1600	56158400	18,3
Total			**306884800**	**100,0**

Tableau 9: Estimation du coût de la lutte contre les vecteurs

Rubrique	Produits	Quantité	Prix unitaire (FCFA)	Coût total (FCFA)	Pourcentage
	Virkon®	20 kg	5000	100000	
	Dursban®	2 litres	25000	50000	
	Propoxur® 2%	50kg	22000	22000	
Achat Désinfectants	Eau de javel, Crésyl®			300000	98,7 %
Carburant	Carburant	10 litres	600	6000	1,3 %
Total				**478000**	**100,00 %**

478 000 FCFA, la presque totalité (98,7 %) étant consacrée à l'achat des désinfectants (tableau 9). La lutte a coûté 478 000 FCFA, la presque totalité (98,7 %) étant consacrée à l'achat des désinfectants (tableau 9).

Le coût de la coordination

Calcul de l'amortissement du matériel

Les frais d'amortissement du matériel utilisé dans la lutte sont évalués à 31 717 296 FCFA (tableau 10).

Tableau 10: Tableau d'amortissement du matériel utilisé dans la lutte

Immobilisation	Nombre	Prix Unitaire (FCFA)	Coût total (FCFA)	Durée d>utilisation		Amortissement (FCFA)
				Années	Mois	
Voitures	19	14500000	275500000	5	60	22958333
Motocyclettes tout terrain	30	1200000	36000000	4	48	3750000
Réfrigérateur électrique	13	292640	3804320	5	60	317027
Congélateurs	16	298540	4776640	5	60	398053
Glacières (15 litre)	172	16538	2844536	3	36	395074
Poste informatique	29	1613300	46785700	3	60	3898808
Total						31717296

Tableau 11: Répartition du coût économique total de la coordination

Rubriques	Montant (F CFA)	Pourcentage du coût économique de la coordination
Seringues, aiguilles, alcool, coton	2 649 000	3 %
Communication/ sensibilisation	25 900 000	30 %
Frais de mission de supervision et des réunions	12 451 000	15 %
Carburant (vaccination et supervision)	11 000 000	13 %
Frais consommation électrique	2 105 892	2 %
Total coûts monétaires de la coordination	**54 105 892**	**63 %**
Amortissement matériel	31 717 296	37 %
Total coût économique de la coordination	**85 823 188**	**100 %**

Coût économique total de la coordination

Le coût total économique de la coordination de la lutte est estimé à 85 823 188 FCFA, avec 37 % pour la dotation à l'amortissement du matériel et 30 % pour les frais de communication et de sensibilisation. Les frais de missions de supervision des réunions et du carburant représentent respectivement 15 % et 13 % des coûts. Les frais d'achat du petit matériel et de consommation électrique représentent respectivement 3 % et 2 % du coût de la coordination (tableau 11).

Coût global de la lutte

Le coût global de la lutte s'élève à 398 328 133 FCFA. Une part importante de ce coût est occupée par la vaccination (77,04 %) et la coordination (21,55 %). Le coût du diagnostic représente 1,29 % et celui de la lutte contre les vecteurs 0,12 % (Tableau 13).

Coûts économiques totaux de la maladie et ratio coûts-avantages

Le coût économique total de l'épizootie 2007 de la peste équine au Sénégal est estimé à 896 790 798 FCFA, avec 55 % pour les pertes par mortalité ou par morbidité, et 45 % pour le coût de contrôle de la maladie (tableaux XII et XIV). Le gain additionnel est de 498 462 665 FCFA tandis que le coût additionnel est estimé à 398 328 133 F CFA , ce qui permet d'obtenir un ratio coûts-avantages de 1,25 sur cinq mois de lutte.

Ces estimations montrent qu'en investissant

Tableau 12: Coûts économiques et ratio coûts-avantages

Rubriques	Coûts économiques (F CFA)	Pourcentage	Coûts/catégorie (F CFA	Pourcentage/ catégorie
Mortalité	493655165	55,05		
Perte force de travail	4807500	0,54	498462665	55,58
Diagnostic	5142145	0,57		
Vaccination	306884800	34,22		
Lutte contre les vecteurs	478000	0,05		
Coordination	85823188	9,57	398328133	44,42
Total	**896790798**	100	**896790798**	100
Bénéfice net			**100134532**	
Ratio Bénéfice-coût			**1,25**	

398 328 133 FCFA pour le contrôle de l'épizootie 2007 de la peste équine au Sénégal, une perte nette de 498 462 665 FCFA aurait été évitée. Cet impact est toutefois influencé par l'action de plusieurs facteurs parmi lesquels il faut signaler le plan de lutte d'urgence mis en place par les autorités sénégalaises. Cet impacte est tout de même sous-estimé parce qu'il n'intègre pas dans son calcul les coûts indirects, difficilement quantifiables, tels que ceux dus à l'annulation des courses, aux retard de vente des chevaux (pour les marchands), etc.

Tableau 13 Coûts des rubriques de la lutte contre l'épizootie de2007

Rubriques	Coûts (X103) F CFA	%
Vaccination	306 884,800	77,04
Diagnostic	5 142,145	1,29
Lutte contre vecteurs	478,000	0,12
Coordination lutte	85 823,188	21,55
Total	398 328,133	100

Il ne faut pas négliger l'environnement socioculturel dans lequel l'éleveur évolue. En effet, le comportement collectif observé dans la société sénégalaise a probablement contribué à limiter l'impact économique de la maladie. Les pratiques d'entraide et de partage de ressources pratiquées au Sénégal en sont des exemples. Les éleveurs ayant perdu leurs chevaux de trait ont dû probablement bénéficier de la mobilisation des autres éleveurs de la communauté pour labourer leurs terres, évitant ainsi l'absence de récolte dont les conséquences auraient pu être désastreuses pour le Sénégal.

L'investissement a produit un résultat positif avec un ratio coûts-avantages de 1,25 sur cinq mois de lutte. Ce ratio est proche de ceux rapportés par TAMBI et coll. (2006) dans un exercice d'estimation de l'impact économique de la PPCB dans quelques pays africains. Ces résultats révèlent que le contrôle des maladies animales est économiquement rentable. Il est donc possible à travers une stratégie de prévention bien menée, par la vaccination et la lutte contre les vecteurs par exemple, de limiter l'impact économique de la peste équine au Sénégal.

Tableau 14: Coûts économiques globaux de l'épizootie de 2007

Rubriques	Coûts en F CFA	%
Mortalité	493 655 165	55,05
Perte force de travail	4 807 500	0,54
Vaccination	306 884 800	34,22
Diagnostic	5 142 145	0,57
Lutte contre vecteurs	478 000	0,05
Coordination lutte	85 823 188	9,57
Total	896 790 798	100

Conclusion

L'étude sur l'impact économique de l'épizootie 2007 de la peste équine révèle que l'épizootie a entraîné 1137 mortalités sur un effectif de 517 614 chevaux estimés soit 0,22 % dans les élevages traditionnels, et 32 morts sur 598 chevaux dans les élevages modernes soit un taux de 5,35 %. Par ailleurs, l'épizootie a entraîné un total de 1357 malades sur un effectif de 517 614 chevaux soit un taux de morbidité national de 0,26 % dans les élevages traditionnels. Il n'a pas été enregistré de mortalités dans les régions du sud du pays (Ziguinchor et Kolda).

Le coût économique total de cette épizootie a été estimé à environ 896 800 000 FCFA. Les pertes liées à la mortalité et à la morbidité représentent la part la plus importante de ce coût avec 498 500 000 FCFA, soit 55,58 % du coût économique total. Le reste du coût économique total (398 330 000 FCFA, soit 44,42 %) est dû au contrôle de la maladie.

Ces résultats attestent que l'investissement fait au début de l'épizootie a produit un résultat positif avec un ratio coûts-avantages de 1,25. Ainsi, si les autorités avaient investi 398 330 000 FCFA pour la prévention de la peste équine, cela aurait permis d'éviter une perte de 498 500 000 FCFA.

Il ressort de cette étude qu'il est donc possible, à travers une bonne connaissance de l'épidémiologie de la maladie, particulièrement celle des sérotypes circulants, une stratégie de prévention bien menée par une vaccination adaptée aux sérotypes et la lutte contre les vecteurs par exemple, de limiter de façon durable l'impact économique de la peste équine au Sénégal. Ainsi, on sait maintenant que plusieurs sérotypes du virus de la peste équine circulent au Sénégal (au moins les sérotypes 2 et 9) et que ne vacciner ses chevaux que contre le sérotype 9 peut ne pas assurer une parfaite protection des animaux contre la peste équine Cette stratégie de prévention serait plus efficace si les pouvoirs publics allouent davantage de moyens au Programme de Développement de la Filière Equine, afin de permettre à ses coordonnateurs, d'une part de mieux sensibiliser les éleveurs sur les risques qu'ils courent en ne vaccinant pas leurs chevaux contre la peste équine et d'autre part de mieux encadrer les éleveurs sur le plan des bonnes pratiques en élevage équin.

Bibliographie succincte

Bourdin P., Laurent A. 1974 Note sur l'écologie de la peste équine africaine. Rev Elev Méd Vét. Pays tropicaux 27 (2) : 163-168

Faye A. 1988 Le rôle des équidés dans le développement rural en zone sahélo-soudanienne du Sénégal ; le cas du cheval dans le Sud du bassin arachidier

CIRAD/MESRU. Economie de la mécanisation en Région Chaude. Montpellier sept 1988

Lefevre P.C., Blancou J. ; Chermette R. 2003 Principales maladies infectieuses et parasitaires du bétail : Europe et Régions chaudes. Tome 1er Ed. TEC et Doc /EM international, Paris 11 rue Lavoisier

Ly Ch., Fall B., Camara B., Ndiaye C.M. 1998
Le transport hippomobile urbain au Sénégal ; situation et importance économique dans la ville de Thiès
Rev. Elev. Méd Vét Pays trop., 51 (2) : 165-172

Tambi N E, Maïna W O, Ndi C. 2006
An estimation of economic impact of contagious bovine pleuropneumonia in Africa.
Rev Sc et tech OIE vol 25 (3) 999-1012

CLINICAL, HAEMATOLOGICAL, BIOCHEMICAL AND PATHOLOGICAL MANIFESTATIONS OF SUB-ACUTE TOXICITY OF *NICANDRA PHYSALOIDES* (L) GAERTN IN CALVES

MANIFESTATIONS CLINIQUES, HEMATOLOGIQUES, BIOCHIMIQUES ET PATHOLOGIQUES DE LA TOXICITE SUBAIGUE DU NICANDRA PHYSALOIDES (L) GAERTN CHEZ LES VEAUX

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Abstract

The plant *Nicandra physaloides* (*solanaceae*) has been widely associated with livestock poisoning in Kenya and elsewhere. The clinical signs reportedly associated with its poisoning in cattle are depression, circling, tremors, tachycardia, bloat, convulsions, coma and death. Although several cases have been suspected, there is no published information on the *toxicology* of this plant in Kenya. In this study the plant's sub acute *toxicity* was determined in calves. Dried ground plant material was fed to calves for 14 weeks during which time blood was collected at weekly intervals for haematological and biochemical analysis in subacute toxicity studies. The treated calves transiently exhibited muzzle drying, *cardiac arrhythmia*, loose feces, and staggering gaits and reduced growth rate. The activity of the enzyme gamma-glutamyltransferase (GGT) and the mean corpuscular volume (MCV) were significantly lower ($P<0.05$) in the treated group. There was no significant difference ($P>0.05$) between the treated and untreated groups in total protein, hemoglobin, red blood cells, packed cell volume, mean corpuscular *hemoglobin* concentration, white blood cells, lymphocytes and neutrophils, aspartate aminotransferase, creatinine and blood urea nitrogen. There were no mortalities, no gross or histopathological lesions in all the groups. The possible reasons for the difference in behaviour between the calves in this experiment and the suspected natural cases were thought to be due to the variations in animal susceptibility or even plant toxin content. It is concluded that the plant *Nicandra physaloides* growing around the Kabete areas of Kenya may contain toxic phytochemicals that may cause poisoning in livestock if consumed. *Nicandra physaloides* is known to contain glycosides, steroids and alkaloids from earlier studies. More studies are necessary to determine the nature of these phytotoxins and their exact mode of action. Meanwhile livestock keepers are advised to control this plant in their pastures and avoid its consumption by livestock.

Key words: *Nicandra physaloides, subacute toxicity,* calves

Résumé

La plante Nicandra physaloides (de la famille des solanacées) a été largement associée à l'empoisonnement du bétail au Kenya et dans bien d' antes pays. Les signes cliniques qui, d'après les études, sont associés à son empoisonnement chez les bovins sont la dépression, la listériose, les tremblements, la tachycardie, le ballonnement, les convulsions, le coma et la mort. Malgré la signalisation, à maintes reprises, des cas suspects d'empoisonnement à Nicandra physaloides, il n'existe pas, à proprement parler, del données concrètes officiellement publiées sur la toxicologie de cette plante au Kenya. Dans le cadre de la présente étude, la toxicité subaiguë de la plante a été expérimentée et confirmée chez les veaux. Pendant 14 semaines, des veaux ont été nourris

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de matières végétales séchées au sol et ont fait l'objet de prélèvements sanguins hebdomadaires aux fins d'analyse des paramètres hématologiques et biochimiques dans le cadre des travaux de recherche sur la toxicité subaiguë. Les veaux soumis au traitement présentaient, pendant un moment, des symptômes d'assèchement du museau, darythmie cardiaque, de diarrhées et de démarche chancelante et affichaient, par ailleurs, un taux de croissance réduit. Le niveau d'activité des enzymes gamma-glutamyl-transférases (GGT) et le volume globulaire moyen (VGM) du groupe de veaux sous traitement accusaient une faiblesse inquiétante (P <0,05). S'agissant de la quantité globale des protéines, de l'hémoglobine, des globules rouges, de l'hématocrite, de la concentration moyenne de l'hémoglobine corpusculaire, des globules blancs, des lymphocytes et des neutrophiles, de laspartate aminotransférase, de la créatinine et de lurée sanguine dazote, aucune différence significative (P> 0,05) n'a été notée entre les groupes de veaux sous traitement et les veaux non traités. Aucun décès n'a été relevé, tout comme aucune lésion macroscopique ou histopathologique n'a été constatée dans les deux groupes. Les raisons possibles de la différence de comportement entre les veaux sélectionnés dans le cadre de cette expérience et les cas suspects procèdent, selon les estimations de l'équipe de recherche, des variations de la sensibilité des animaux, voire, de la teneur en toxine des plantes. Ce constat permet de conclure que la nicandre faux-coqueret (connue sous l'appellation scientifique de Nicandra physaloides) qui pousse dans les alentours de Kabete au Kenya contient probablement des substances phytochimiques toxiques en mesure de provoquer des intoxications chez le bétail en cas de consommation. Les précédents travaux de recherche menés sur la nicandre faux-coqueret (nicandra physaloides) ont permis de découvrir que cette plante contient des glycosides, des stéroïdes et des alcaloïdes. La détermination de la nature de ces phytotoxines et de leur mode daction exact nécessite des études plus approfondies. Entre temps, il est fortement recommandé aux éleveurs de surveiller la présence de cette plante dans leurs pâturages et déviter sa consommation par le bétail.

Mots clés: nicandra physaloides ; toxicité subaiguë ; veaux

Introduction

Poisoning of man and livestock by plants has been known to mankind since time immemorial and continues to pose great challenges and enormous economic losses to the livestock industry in many parts of the world (Nielsen, 1988; James *et al.,* 1992). In the United States of America it was estimated that plant poisoning on rangelands cost producers and consumers upwards of $250 million annually (Nielsen, 1988). There are no published estimates of such economic losses in Kenya but the aggregate of the losses must run into millions of shillings each year. Furthermore, many weeds, which occur in cultivated and pasture land, are thought to be poisonous under certain circumstances. Some plant compounds such as cardiac *glycosides* are toxic to man and animals when ingested in large quantities but are well known medicines at lower doses (Huffman, 2003). Generally, ruminants differ from non-ruminants in their response to many plant toxins because of the action of the rumen microbes. For example, the toxicity of plant toxins like oxalates, mimosine and heliotrine are reduced by rumen fermentation whereas that of cyanogenic glycosides and formononetin (an estrogenic substance found in some plants) is increased (Cheeke, 1995). Furthermore, sheep and goats display greater resistance to poisonous plants than cattle probably as a reflection of their feeding strategy and nature of the diet upon which the species have evolved (Van Soest, 1987).

The plant *Nicandra physaloides* or the "apple of Peru" grows as a weed in cultivated pasture land and is recognized and claimed to be poisonous to livestock by farmers in the highland areas about 1700 to 2700m above sea level in Kenya (Agnew, 1974). In Kenya, several cases of suspected Nicandra physaloides poisoning (always during the wet season) have been reported to the large animal clinic of the

University of Nairobi (unpublished report). Two such incidences involving zero-grazed dairy cattle were investigated and mortality rates found to be 10 and 27% prior to this study. In the above cases, the symptoms occurred 6 to 8 hours after feeding on chopped Napier grass contaminated with succulent Nicandra physaloides. The clinical signs observed in the cases reported at the University of Nairobi large animal clinic included depression, circling, tremors of the hind limbs, fast heart rate, ruminal stasis and bloating, convulsions, coma and death. An acute toxicity study in mice gave intra-peritoneal LD50 values of 1.86, 2.58 and 3.65 g/kg body weight in leaf, fruit and whole plant extracts, respectively (unpublished). However, no other detailed studies on the toxicity of Nicandra physaloides have been carried out in Kenya. The purpose of this study therefore, was to investigate the sub-acute toxicity of Nicandra physaloides following experimental dosing of calves with ground dried plant material in feed. Clinical signs of poisoning and the effects of the plant on haematological, biochemical and pathological aspects were investigated.

Materials and methods

Study area

The mature plant materials of *Nicandra physaloides* (L) Gaertn were collected from around Kabete (approximately 1°16'S, 36°43'E) and Kikuyu (approximately 1°15'S, 36°40'E) areas, both corresponding to the location of the affected dairy herds. These areas lie about 20 kilometres west of the Kenyan capital city of Nairobi, at altitudes of around 1900m above sea level and annual rainfall of between 800-1200mm falling in a bimodal pattern.

Collection, identification and preparation of the plant

The collection was done by cutting the mature plant about one foot from the soil level and then transporting it whole to Kabete campus. The plant material was positively identified at the herbarium section of the National museums of Kenya and voucher specimen deposited. The plant material (stems, leaves, flowers and fruits) was left to dry in the shade and then ground into fine powder using a MM 20 grinder from Marina Machineries Ltd, Nairobi, Kenya.

Experimental animals

Ten clinically healthy bull calves of the Friesian breed, aged 8 to 10 months, were bought from different farms around Kabete area and transported for housing in individual pens at the large animal unit of the Department of Clinical Studies, University of Nairobi. The calves were identified by use of labeled ear tags, weighed and dewormed using an Albendazole (Valbazen®, Kenya Swiss Ltd) at the dosage rate of 10 mg/kg body weight on arrival and one month later. The calves were put on a diet of grass hay and wheat bran, and supplied with mineral lick and water *ad libitum* and allowed to acclimatize to the new environment for two weeks. All requirements on ethics in animal experimentation were met and adequate measures taken to minimize pain and discomfort to all the animals used in this study.

Experimental design

The calves were randomly assigned to 5 groups, A to E (n=2) which were further randomly assigned to 4 treatment groups and one untreated control. Pretreatment blood samples for haematology (5ml with EDTA anticoagulant) and biochemistry (15ml without anticoagulant), body weights and vital physiological parameters (rectal temperature, heart and respiratory rates) were measured to obtain baseline data for each animal. Bleeding was aseptically done from the jugular vein using hypodermic needles gauge 18 (1.2 x 40mm). The blood without anticoagulant was allowed to clot at room temperature and the serum separated by centrifugation at 2300 g for 10 minutes. The serum was stored at 4°C when used on the same day or frozen at

-20°C when used later. Hematological and biochemical analyses were done using standard procedures.

Treatment groups B, C, D and E were fed on wheat bran mixed with ground whole Nicandra physaloides powder at 4, 8, 16 and 32% w/w respectively, for 14 weeks while the control group A was fed on wheat bran alone. The daily mixed ration (wheat bran and / plant material) for each calf was 2kg. All groups were also given grass hay, mineral lick and water *ad libitum* throughout the experiment. The health status of each calf was monitored on a daily basis by recording rectal temperature, ruminal motility, heart and respiratory rates. The body weight of each calf was recorded once every 4 weeks for the 14 weeks of study. The blood for hematology and biochemistry analyses was taken pre-treatment and on weeks 1, 2,3,4,5,6,9,11,13 and 14 post-treatment and analyzed using routine procedures (Stockham and Scott, 2002; Kaneko *et al.*, 1997). At the end of the 14 weeks treatment period, all calves were euthanized humanely and systemic gross and histo-pathological examination carried out using routine techniques at the Department of Veterinary *Pathology*, *Microbiology* and *Parasitology*, University of Nairobi.

Statistical Data Analysis: The hematological and serum biochemical data were analyzed using the t-test and analysis of variance (Genistat® 9th edition). The results were then presented in the form of tables and line graphs.

Results

Clinical Manifestations of sub-acute Nicandra physaloides in Calves

All the experimental calves had varying degrees of loose feces and dry muzzle between day 2 and 3 post-treatment which disappeared on day 5. Fifty percent (50%) of calves fed on the plant material had irregular heart beats on the 4th day post-treatment. Fifty percent of calves fed 8% w/w N. *physaloides* had staggering gaits (during the weighing exercise) on the first week post-treatment. The calves fed on the plant material at the concentrations of 16% w/w and above were initially reluctant to take the feed, but got used to it by the

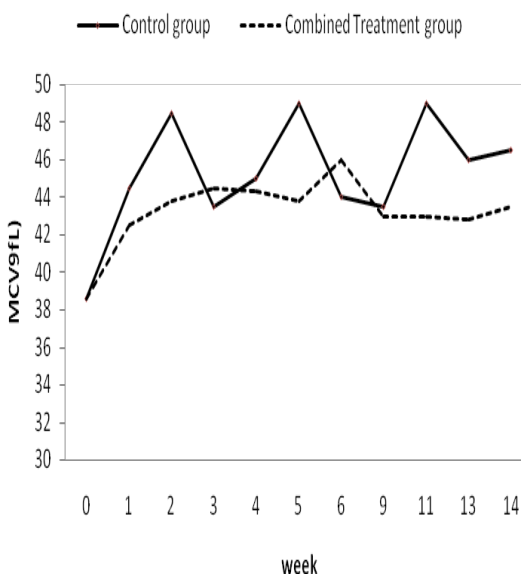


Fig. 1: MCV (fL) TRENDS in the control versus the combined treatment group

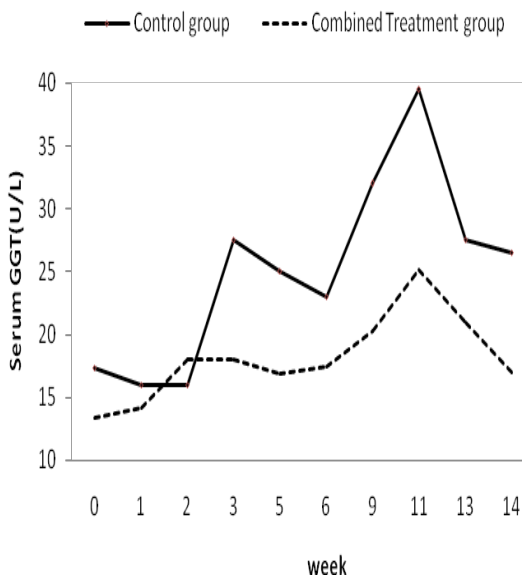


Fig. 2: Serum GGT (U/L) in the control versus the combined treatment group

Table 1: Effects of *Nicandra physaloides* on selected blood parameters in calves

Blood Parameter	Units	Pretreatment means	Treatment means	Normal range (Bovine) §	Comments
GGT	U/L	14.2	19.5	4.9-25.7	Increase*
AST	U/L	36.2	48.4	45.3-110.2	Increase*
Creatinine	mg/dL	2.1	2.7	0.6-1.8	Increase*
BUN	mg/dL	6.7	10.4	7.8-24.6	Increase*
Total protein	g/dL	6.6	6.5	6.2-8.2	No change
Hemoglobin	g/dL	7.6	8.9	8-15	Increase*
RBC	$X10^{12}$ /L	6.8	6.9	5-10	No change
PCV	%	26.3	26.6	24-46	No change
MCV	fL	38.6	43.6	40-60	Increase*
MCHC	X10 g/L	28.8	33.2	30-36	Increase*
WBC	$X10^{9}$ /L	8.3	8.3	4-12	No change
Lymphocytes	$X10^{9}$ /L	5.9	5.6	2.5-7.5	No change
Neutrophils	$X10^{9}$ /L	2.3	2.6	0.6-4	No change

*Significant increase at $p < 0.05$

§References for normal bovine parameters obtained from the Merck Veterinary Manual, seventh edition (1991) pp 965 – 970. Merck & Co., Inc. Rahway, N.J., U.S.A

second week post-treatment. Although all the experimental and control calves gained weight of between 13 to 30% during the 14 weeks experimental period, it was observed that the weight gains decreased with increasing concentrations of *N. physaloides* in the feed.

Effects of Nicandra physaloides sub-acute toxicity on the Hematological Parameters in Calves

The comparison between the pretreatment and the treatment values for the various hematological parameters are shown on Table 1. The levels of hemoglobin, MCV and MCHC were significantly higher 14 weeks post-treatment compared with the pretreatment values ($P<0.05$). The MCV was significantly lower (relatively microcytic) in the treatment group than in the control (Fig.1).

Effects of sub-acute toxicity Nicandra physaloides on the Serum Biochemical Parameters in Calves

The pretreatment and treatment means of the various biochemical parameters are shown in Table 1. The levels of the serum enzymes (GGT and AST) and metabolites (BUN and creatinine) assayed during the experimental period were significantly higher ($P<0.05$) than the pretreatment levels in all the groups of animals including the control, but remained within the normal references for cattle. Creatinine was an exception in that the pretreatment and treatment values for all groups tended to be higher than the normal references for cattle. The *serum* GGT activities were significantly lower ($P<0.05$) in the combined treatment group than in the control (Fig. 2). There were no significant differences in the serum levels of AST, BUN and Creatinine between the control and the combined treatment groups.

Gross and Histo-pathological examination

There were no obvious gross or *histopathological* changes noted in all the groups of calves.

Discussion and Conclusion

The signs of dry muzzle and loose

feces in the calves during the first week of the experiment are suggestive of mild gastrointestinal irritation and discomfort. The lower weight by the calves fed on *Nicandra physaloides* could have been as a result of reduced nutrient intake or direct toxic effect by the plant. The irregularity in the heart beats and the staggering gait observed in some of the calves, albeit brief, could suggest a central nervous involvement. Some plants of the *Solanaceae* family, especially those of the solanine group with glycosidic steroidal alkaloids, are known to cause gastrointestinal irritation, central nervous system stimulation and depression of cardiac and respiratory centres (Clarke, 1981; Radostits *et al.,* 2000). Furthermore, *Nicandra physaloides* is rich in glycosides, steroids and alkaloids (Begley *et al.,* 1976) and these could be responsible for the mild signs that were observed.

The calves showed only mild clinical signs and no deaths occurred as reported in literature (Cohen, 1970, Abebe *et al*., 2001 and Radostits *et al*., 2000). This could be as a result of variations in animal susceptibility or even plant toxin content. It is a fact that animals may respond differently to poisoning by the same plant depending on the dose, stress or even other plants they may be ingesting (Hooper, 1978). Perhaps the drying process denatured and reduced the effects of toxins in the plant. This is likely so because the calves in this experiment were always on dry fibrous feed making them less prone to bloat unlike in the case reported in sheep by Cohen (1970) and those seen earlier in dairy cattle in the field (personal observation). Most of the suspected natural cases are usually on green and often lush fodder. It is also known that toxicity of plants to livestock may vary with their stage of growth, treatment, time of the year, habitat, and the weather and seasons (Mandhar, 2002; Radostits *et al*., 2000). Furthermore, Begley *et al*., (1976) found that the product mixture of the aqueous extract from *Nicandra physaloides* varied between crops suggesting a possible relationship with climate, time of harvesting and habitat.

The overall increase in the body weights and some of the assayed blood parameters (hemoglobin, MCV, MCHC, BUN, AST, GGT and creatinine) in all groups of calves including the control could have been as a result of the natural growth process since age has a major effect on several parameters (Kaneko, 1997; Otto *et al*., 2000). This could be more so because the parameters generally remained within the normal ranges for bovine references except for creatinine values which were generally higher, for both the control and treatment groups. Since most of the published references are from exotic breeds it is possible that creatinine values for cattle in this area are higher than those reported elsewhere.

The reason for the significant difference in the values of GGT and MCV between the treatment and the control groups is not very clear and can only be matter of conjecture. Gamma-glutamyltransferase is a cell surface *glycoprotein* that cleaves *C-terminal glutamyl* groups from *glutathione* and *glutathione* conjugates and transfers them to (acceptor) peptides and *L-amino* acids (Lieberman *et al.,* 1995; Hanigan and Pitot, 1985) and in this regard plays an important role in the transport of amino acids into the cells. The molecular mechanism by which iron is *physiologically* transported through the cellular membranes is only partially understood, but several studies indicate that a reduction step from ferric to ferrous state is necessary in both transferrin mediated and transferrin independent iron uptake (Walker *et al*., 2001). Recent studies (Dominici *et al.,* 2003) have described GGT as a factor capable of effecting the reduction of iron from ferric to ferrous state in the cell microenvironment. Most tissues have GGT activity with kidneys having the highest and muscles the least. However, most serum GGT is derived from the liver (Braun *et al*., 1978). Decreased levels of GGT can be found in *hypothyroidism, hypothalamic*

malfunction and low levels of *magnesium*. Decreased hepatic synthesis due to severe viral hepatitis and extensive liver cirrhosis can also lead to a decline in serum GGT activity (Rutenburg *et al.,* 1963; Villa *et al*., 1966). Known inhibitors of GGT like acivicin and serine-borate complex can also lead to decreased activity (Tate and Meister, 1978). Fully expressed iron deficiency anemia is usually microcytic hypochromic but may be preceded by microcytic normochromic or normocytic hypochromic red cells (Dallman, 1977; Harvey, 2006). The relative microcytic normochromic behaviour in the treatment versus the control groups in this study could possibly have been as a result of GGT inhibition and therefore decreased uptake of iron into the hemopoietic cells, thus creating an artificial iron deficiency. This apparent shortage of iron to the cells and possibly an interference with the amino acid uptake could have interfered with hemoglobin synthesis in the calves fed on *Nicandra physaloide*s. Probably be the fully expressed anemic picture with other accompanying changes and signs could have developed if the feeding was continued for a longer period of time.The general downward trend, in the treated group, of GGT, RBC, PCV, *hemoglobin* and total proteins towards the end of the experiment further supports this position. Further work is necessary to investigate the effects of dosing the animals for a longer duration and using green plant material at different stages of growth. The real nature of the *phytotoxins* and their exact mode of action also need to be fully elucidated.

Impact

This is the first comprehensive study to investigate the toxic effects of the plant *Nicandra physaloide*s to livestock. The results indicate that this plant is potentially poisonous to livestock and therefore, great caution should be taken to avoid its consumption by livestock. This plant grows abundantly in cultivated areas contaminating pasture and fodder crops and could therefore, be easily accessible to livestock and cause immense losses. Further studies are necessary to determine the nature of the bioactive compounds in this plant and their possible utilization in the pharmaceutical industry because of the well known relationship between poisons and drugs.

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References

Abebe D, Urga K, Debella A, Ambaye C and Dejene A. 2001. A survey of Poisonous plants in southern Ethiopia. *Ethiopian Journal of Health Development* 15: 139-144

Agnew A D, 1974. Upland Kenya wild flowers. Oxford University Press. U.K. p.529.

Begley M J, Crombie L, Ham P J and Whiting D A (1976). Structures of three oxygenated 24-methyl-steroids (Nic-3, 7 & 11) from the insect repellent plant *Nicandra physaloides* (Solanaceae). *Journal of the Chemical Society, Perkin Transactions* I: 296-304.

Braun J P, Rico A G, Bernard P, Thouvenot J P and Bonnefis M J. 1978. Tissue and Blood distribution of *Gamma-glutamyltransferase* in the lamb and in the ewe.*Research in Veterinary Science* 25: 37- 40.

Cheeke P R. 1995. Endogenous *toxins* and *mycotoxins* in forage grasses and their effects o n

livestock. *Journal of Animal Science* 73:909-918.

Clarke M L. 1981. In: Veterinary Toxicology 2nd edn. Ed: Clarke, M.L, Harvey, D.J and Humphreys, D.J. Bailliere Tindall, London.

Cohen R D. 1970. Bloat in sheep grazing wild gooseberry, *Nicandra physaloides*. *Australian Veterinary Journal* 46: 559.
Dallman P R. 1977. New approaches to screening for iron deficiency. *Journal of Pediatrics* 90: 678.

Dominici S, Pieri L, Comporti M and Pompella A. 2003. Possible role of membrane *gamma-glutamyltransferase* activity in the facilitation of transferring dependent and independent iron uptake by cancer cells. *Cancer Cell International* 3: 7.

Hanigan M H and Pitot H C. 1985. *Gamma-glutamyltranspeptidase* and its role in *hepatocarcinogenesis*. *Carcinogenesis* 6: 165-172

Harvey J W. 2006. *Microcytic Anemias*. In: Schalm's Veterinary *Hematology* 5th ed. Bernard, V F, Joseph G Z and Nemi C J. *Blackwell Publishing Professional*, 2121 State Avenue, Ames, Iowa 50014, USA.

Hooper P T. 1978. *Pyrolizidine alkaloid* poisoning-*pathology* with particular reference to differences and plant species. In: Keeler, R.F., Van Kampen, K.R and James, L.F eds). Effects of poisonous plants on livestock. Academic Press, New York.

Huffman M A. 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants". Proceedings of *Nutraceutical* Society 62: 371-81

James L F, Nielsen D B and Painter K E, 1992. Impact of poisonous plants on the Livestock industry. *Journal of Range Management*. 45:3-8.

Kaneko J J, Harvey J W and Bruss M L, 1997. *Clinical biochemistry of domestic animals,* 5th ed. Academic Press, San Diego, USA.

Lieberman M W, Battios R, Carter B Z, Habib G M, Shi Z and Wan D, 1995. Gamma-Glutamyl-transpeptidase: what does the organization and expression of a multipromoter gene tell us about its functions? *American Journal of Pathology* 147: 1175-1185

Manandhar N P 2002. *Plants and people of Nepal*, Timber press. Oregon .

Nielsen D B, 1988. Economic impact of poisonous plants on the rangeland livestock industry. *Journal of Range Management* 66: 2330-3.

Otto F, Vilela F, Harun M, Taylor G, Baggasse, P and Bogin E. 2000. *Biochemical* blood profile of Angoni cattle in Mozambique. Israel *Journal Veterinary Medicine* 55(3).

Radostits O M, Gay CC, Blood D C and Hinchcliff K W, 2000. Diseases caused by
major *phytotoxins*. In: *Veterinary Medicine*, 9th ed. W.B. Saunders, London, New York, Philadelphia, San Francisco, St Louis, Sydney.

Rutenburg A M, Goldbarg J A and Pinenda E P, 1963. Serum *gamma-Glutamyltranspeptidase* activity in or pancreatic disease. *Gastroentorology* 45: 43-48.

Stockham S L and Scott M A, 2002. Fundamentals of *Veterinary Clinical Pathology*, 1st ed. Blackwell publishing professional, Ames, Iowa, USA.

Tate S S and Meister A, 1978. Serine-borate complex as a transition-state inhibitor of
gamma-glutamyltransferase. *Proceedings of the National Academy of Sciences* U.S.A 75: 4806-9.

Van Soest P J, 1987 Nutrition Ecology of the Ruminant, Cornell University Press, Ithaca, New York, USA.

Villa L, Dioguardi N, Agostoni A, Ideo G and Stabilini R, 1966. Prognostic value Of serum *gamma-glutamyltransferase* activity in liver disease. *Enzymologia Biologia* et Clinica, 7: 109-114.

Walker B L, Tiong J W, Jeffries W A, 2001. Iron metabolism in mammalian cells. *International Reviews on Cytology*. 211: 241-278.

Influenza A antigen, Newcastle and Gumboro Diseases Antibodies in Apparently Healthy Local Poultry

Antigènes de la grippe A, anticorps des maladies de Newcastle et de Gumboro constatés chez la volaille locale apparemment en bonne santé

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Key words: Apparently healthy, Avian influenza, Gumboro disease, Kaduna State, Local poultry, Newcastle disease,

Abstract

The study assessed prevalence of influenza A antigen and antibodies to avian influenza, Newcastle and Gumboro diseases in apparently healthy local poultry in Kaduna State, Nigeria. Seventy-two pooled cloacal and oro-pharyngeal swabs and 264 blood samples were collected from local poultry. Swabs were analysed by reverse transcriptase polymerase chain reaction; sera by agar gel immunodiffusion and haemagglutination inhibition tests. Avian influenza antigen and antibodies prevalence were 1.4% and 0 %, respectively. Mean antibody titre and prevalence of Newcastle disease in chickens were 2.74 ± 0.28 $\log_2$ and 48.5 % respectively. About 68.7 % of chickens had antibody titre <4 $\log_2$ with 42.9 % having titre ≥ 7 $\log_2$. Newcastle disease prevalence rates in ducks and turkeys were 47.8% and 66%, respectively. All local poultry were negative for avian influenza but 55.3 % had antibodies to Gumboro disease. Influenza A virus was circulating among ducks in Dan Gaiya village. Local poultry are exposed to continuous challenge from fowl pox and Gumboro diseases and are highly susceptible to Newcastle disease which can be mistaken for highly pathogenic avian influenza. Continuous active surveillance of avian influenza in local poultry is necessary. Avian influenza control in local poultry should incorporate Newcastle and Gumboro diseases vaccination.

Key words: Influenza A, Newcastle, Gumboro, antigen antibodies PCR

Résumé

L'objectif de la présente étude était d'évaluer la prévalence des antigènes de la grippe A et des anticorps de la grippe aviaire, des maladies de Newcastle et de Gumboro chez la volaille locale apparemment en bonne santé de l'Etat de Kaduna, au Nigeria. Soixante-douze prélèvements cloacaux et de l'oropharynx mis en commun et 264 échantillons sanguins ont été recueillis à partir de la volaille locale. Les prélèvements ont été analysés par la réaction polymérisation en chaîne par transcriptase inverse, par les sérums par immunodiffusion en gélose et par les tests d'inhibition de l'hémagglutination. Les prévalences d'antigènes et d'anticorps de la grippe aviaire ont été de 1,4% et 0% respectivement. Le titre d'anticorps moyens et la prévalence de la maladie de Newcastle chez les poulets étaient de 2,74 ± 0,28 log2 et de 48,5% respectivement. Les résultats de l'analyse ont confirmé que près de 68,7% de l'ensemble des poulets présentaient un titre d'anticorps <4 log2 et que 42,9% avaient un titre d'anticorps moyens ≥ 7 log2. Les taux de prévalence de la maladie de Newcastle chez les canards et les dindes étaient de 47,8% et 66% respectivement. Toutes les races locales de volaille qui ont été soumises au test de la grippe aviaire ont été trouvées négatives, mais 55,3% d'entre elles présentaient des anticorps de la maladie de Gumboro. Le virus de la grippe

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A circule parmi les canards du village de Dan Gaiya. La volaille locale est exposée au problème permanent de la variole aviaire et des maladies de Gumboro et est très sensible à la maladie de Newcastle que l'on peut facilement confondre avec la grippe aviaire hautement pathogène. La lutte active et permanente contre la grippe aviaire chez la volaille locale est nécessaire. La lutte contre la grippe aviaire chez la volaille locale doit en effet intégrer la vaccination contre les maladies de Newcastle et de Gumboro.

Mots clés: Anticorps contre les antigènes de la grippe A ; maladies de Newcastle et de Gumboro ; réaction polymérisation en chaîne (RPC)

Introduction

Local poultry (LP) comprising more than 80 % of poultry population in Nigeria represents a biodiverse rare gene pool and is a source of livelihood to villagers as it produces the bulk of poultry meat and eggs source which might be lost to highly *pathogenic avian influenza* (HPAI) (Abdu et al., 1992). They are raised either extensively or semi-intensively and are exposed to continuous challenge to pathogens such as Newcastle disease (ND), Gumboro disease (IBD) and fowl pox (FP) which are major constraint to LP production (Nwosu, 1997). These LP need to have a robust immune system which confers them the ability to withstand challenge from continuous exposure to field pathogens (Easterday *et al.*, 1997).

The outbreak of HPAI H5N1 in Nigeria in 2006 increases the risk of LP loss to disease especially with reports of highly pathogenic H5N2 virus in healthy water fowls and H5N2 antibodies in apparently healthy local chickens in Northern Nigeria and Kaduna State, respectively (Durosinlorun, 2008; Nicolas *et al.*, 2008). These extensively raised LP with minimal or no biosecurity, are at risk of exposure to ND and AI viruses from direct contact with infected wild birds or indirect contact with fomites (Swayne and Jackwood, 2008). Since LP can maintain these viruses within their production system, they constitute a critical control point in the control of ND, IBD and AI in Nigeria. As such, there is need to assess the LP health, detect influenza A antigen if circulating among LP as suggested by Durosinlorun (2008). The information generated is important in designing a comprehensive AI control program in LP.

Materials and Methods

Study area

Kaduna State consists of 23 LGA with an estimated population of 6 million people and poultry population of 2,821,092 with about 90% being local poultry (RIM, 1993).

The study area involved 11 villages where H5N2 antibodies in local chickens were reported by Durosinlorun (2008). The villages were Ikara town, Sabon garin Jibis, Gidan Shawai, Nasarawa, Saminaka, Yar Kasuwa, Ungwan Sarki, Ungwan Shitu, Sarbon Gari Kargako, Tudun Wadan Kargoko and Dan Gaiya. A village was identified as an *epidemiologic* unit.

Data collection

A structured questionnaire was administered to consenting local poultry farmers prior to sample collection. The questionnaire provided information on farmers' demographic data, flock management, flock disease and vaccination history.

Sample collection

Samples were collected between August and September, 2009. Local poultry were selected without replacement noting their age, sex and any abnormal condition prior to sample collection.

Oropharyngeal and *cloacal swabs* were collected by gently swabbing the oropharynx

and cloacae of live bird of selected poultry species with a sterile dry cotton swab. The swabs were pooled (five swabs per tube) with swab samples of like specimens of the same species from the same village. Swabs were placed in viral transport medium, immediately preserved in a flask with ice packs and transported to and frozen at –83°C at *Avian Influenza* Laboratory NVRI, Vom, until analysed. Two millilitre of blood collected through brachial vein of poultry using 21 G sterile hypodermic needles and 2 ml syringes carefully observing asepsis were allowed to clot at room temperature and sera obtained were stored at – 20^0C until used for serology.

Processing of swabs

Oropharyngeal and cloacal swabs were thawed, vortexed and allowed to settle for 30 minutes at room temperature. The swab was used as a filter for cloacal samples and the filtrate collected into a 1.5 ml tube. The filtrates were stored at – 83^0C until required for RNA extraction (OIE, 2009).

RNA isolation and virus detection.

Viral RNA was extracted using Qiagen QI AampR viral RNA mini kit according to manufacturer's instructions. The extracted RNA were reverse transcribed and amplified a 240 bp fragment of avian influenza genome in a one-step RT-PCR assay using a Forward and Reverse primers sequence for the matrix (M) gene, 5' – *CTTCTAACCGAGGTCCAAACG – 3'* and *5'–AGGGCATTTTGGACAAAKCGTCTA – 3',* respectively. The RT-PCR was performed with Gene AmpR Gold RNA PCR core kit (Applied *Biosystems*, CA- USA) according to manufacturer's instructions in a Gene AmpR thermo cycler PCR system 9700 (Applied Biosystem, CA) and amplified amplicons were detected by gel *electrophoresis*.

All RT-PCR positive samples were inoculated into two 11 day-old specific antibody negative (SAN) embryonated chicken eggs through allantoic cavity and viral isolation performed as recommended by OIE (2009).

Detection of avian influenza antibodies by AGID test

Chicken and turkey sera were screened for avian influenza antibodies using AGID test. The test antigen used was an inactivated H5N2 matrix (M)–antigen while the positive serum was an H5N3 serum both prepared by Istituto Zooprofilattico OIE/FAO Laboratory for AI and NDV delle Venezie. The AGID test was performed as recommended by OIE with incubation at 37^0 C in a humidified incubator and examined after 24 hours (OIE, 2009).

Detection of avian influenza antibodies by HI test

An alpha *haemagglutination inhibition* (HI) test was performed on the duck and geese sera using standard procedures recommended by OIE (OIE, 2009).

The test antigen used was an inactivated H5 subtype–antigen while the positive serum was an H5N2 serum both prepared by Istituto Zooprofilattico OIE/FAO Laboratory for AI and NDV delle Venezie.

Determination of Newcastle disease antibodies titre

Newcastle disease vaccine (La Sota strain) obtained from the NVRI, Vom, Nigeria was used as antigen for the test while the positive sera used as control was obtained from the NVRI Avian Virology Laboratory. An alpha haemagglutination inhibition (HI) test was performed on all poultry sera using standard procedures recommended by Allan and Gough (1974).

Detection of Gumboro disease antibodies

Gumboro antigen was derived from affected bursae of chicken that died from natural IBD. The bursae was removed, diluted 1:1 (w/v) with PBS (pH 7.6); ground with pestle and mortar and centrifuged. The supernatant was extracted and used as antigen. A positive control and a known negative antiserum were incorporated in each test run. The AGID test for Gumboro disease was performed as recommended by

(Harai *et al.,* 1972).

Data analysis

Data generated were analysed by descriptive statistics using Statistical Package for Social Sciences (SPSS) version 17 program.

Results

Disease history

Three weeks prior to the study, farmers in Gidan Shawai experienced chicken mortality with signs of greenish – yellowish diarrhoea, drooling discharge from the mouth and nostrils with most farmers selling their sick poultry. In Saminaka, poultry mortality was mainly from poisoning after feeding on insecticide treated maize while in Yar Kasuwa, there were reports of mortality in ducklings due to blindness while slaughtered ducks had enlarged livers.

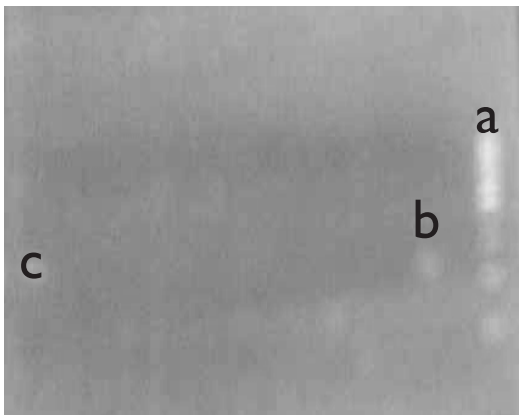


Plate I: RT- PCR *amplicons* of duck cloacal swab from Dan Gaiya village Kaduna State (a = molecular weight marker; b = test sample; c = positive control).

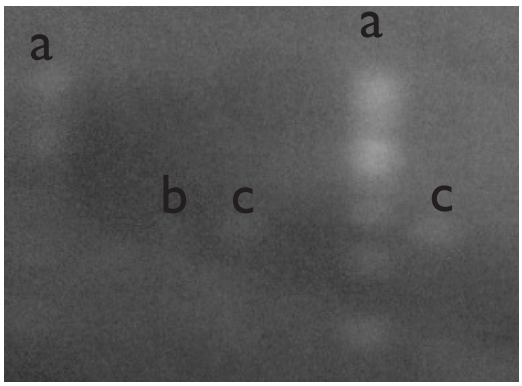


Plate 2: Third RT-PCR screening of duck cloacal swab from Dan Gaiya village Kaduna State (a = molecular weight marker; b = test sample; c = positive control).

Clinical findings

Chickens in Saminaka, Kachia, Tudun Wadan and Sabon Garin Kagarko had pox lesions on the comb, wattles and oral pox lesions in young birds. The prevalence of clinical pox among the villages sampled was 36.4%. Other conditions observed in chickens were scaly legs in Tudun Wada; dehydrated, mud soiled beaks and weak chickens at Unguwan Sarki.

Detection of Influenza A antigen

Seventy–two pooled samples were screened by RT – PCR comprising 12 ducks, two geese, two guinea fowls, two pigeons, five turkeys and 38 chicken oropharyngeal swabs; 2 geese and 13 duck cloacal swab samples making a total of 360 local poultry sampled.

Out of the 72 pooled samples screened, one duck cloacal sample from Dan Gaiya (Plate I) and one chicken oropharyngeal sample from Yar Kasuwa (Plate II) were positive. Based on standard procedure of the NVRI Avian Virology Laboratory, all positive RT-PCR samples are re-screened to ensure reliability of results. Re-screening of the positive duck and chicken samples from Dan Gaiya and Yar Kasuwa gave a negative result necessitating a third RT-PCR screening of the two samples. The samples were simultaneously inoculated into embryonated eggs for viral isolation. On the third RT-PCR screening, the Yar kasuwa chicken sample was negative while the Dan Gaiya duck sample was positive (Plate III). The positive result gave a prevalence rate for influenza A virus of 1.4%.

There was *embryo* death after two days

post inoculation of the positive duck sample from Dan Gaiya but allantoic fluid did not agglutinate chicken RBC (red blood count) by haemagglutination test. Similarly, there was embryo death upon second inoculation of the duck sample without agglutination of chicken RBC (red blood count). However, Yar Kasuwa chicken samples did not cause embryo death on seven days post inoculation with no *agglutination* of chicken RBC. Similar result were obtained on second inoculation into *embryonated* eggs.

Avian influenza antibodies

All local poultry sampled in all the sampled villages were negative for avian

Table 1: Sero-prevalence of Newcastle disease and Gumboro disease in local chickens various villages sampled in Kaduna State, Nigeria.

Village	ND prevalence (%)	IBD prevalence (%)
Unguwan Sarki	12 (57.1)	13 (54.2)
Dan Gaiya	0 (0.0)	4 (66.7)
Gidan Shawai	10 (62.5)	9 (52.9)
Ikara Town	4 (36.4)	7 (43.8)
Kachia	5 (71.4)	3 (50.0)
Saminaka	11 (35.5)	23 (36.8)
Sabon Garin Kargako	8 (42.1)	9 (50.0)
Sabon Garin Jibis	8 (50.0)	13 (65.0)
Tudun Wada Kargako	8 (60.0)	16 (61.5)
Yar Kasuwa	10 (62.5)	8 (50.0)
Unguwa n Shitu	5 (55.6)	7 (77.8)
Overall	**83 (48.5)**	**112 (55.3)**

Table 2: Newcastle disease mean antibody *titre* and chicken population with mean *titre* < 4 log2 and ≥7 log2 in sampled villages in Kaduna State

Village	Mean ND antibody titre ± S.E (log2) antibody titre	Chicken with ND antibody titre ≥ 4 log2 (%)	Chicken with ND ≥ 7 log2 (%)
Unguwan Sariki	3.33 ± 0.88	7 (33.3)	5 (41.7)
Dan Gaiya	0 ± 0	0 (0)	0 (0)
Gidan Shawai	3.69 ± 0.95	8 (50.0)	5 (50)
Ikara Town	2.09 ± 1.07	3 (28.9)	2 (50)
Kachia	5.14 ± 1.87	4 (57.1)	3 (60)
Saminaka	2.06 ± 0.66	7 (22.6)	5 (45.5)
Sabon Garin Kargako	3.11 ± 1.01	5 (26.3)	5 (62.5)
Sabon Garin Jibis	3.27 ± 0.98	6 (37.5)	4 (50)
Tudun Wada	1.42 ± 0.53	3 (15.0)	1 (12.5)
Yarkasuwa	4.06 ± 0.96	8 (50.0)	4 (40)
Unguwan Shitu	3.11 ± 1.33	3 (33.3)	3 (60)
Overall	**2.74 ± 0.28**	**54 (31.3)**	**37 (42.9)**

influenza type *A antibodies* by either AGID test for chicken and turkey or HI test for duck and geese sera. Hence prevalence of *AI antibody* was 0 % by both AGID and HI tests.

Newcastle disease antibodies

Ten of the 11 (90.9 %) villages sampled had chickens that were positive for antibodies against ND by HI test with an overall mean HI antibody titre of 2.74 ± 0.28 log_2. The prevalence of ND antibodies of chickens sampled was 48.5 % with 31.3 % having HI antibody ≥ 4 log_2. However, in most villages, more than 50 % of chickens positive for ND antibodies, had antibody titre ≥ 7 log_2. The prevalence of individual villages, together with their mean HI antibody titre and percentage of chickens with the ND titre ≥ 4 log_2 are presented in Table 1 and Table 2 respectively. The mean ND HI titre of hens and cocks was 3.58 ± 0.5 log_2 and 2.81 ± 0.56 log_2 respectively. However, the mean HI titre of adult chickens was 3.14 ± 0.37 log_2 while that of growers was 2.06 ± 0.41 log_2. There was an association (p = 0.042) between chicken age and ND antibody titre (Chi_2 = 16). Though the mean ND titre of ducks sampled was 2.78 ± 0.69 log_2 with a prevalence of 47.8 %, the HI titre of adult and young ducks were 3.26 ± 0.79 log_2 and 0.50 ± 0.05 log_2,respectively. However, 43.5 % of ducks had HI titre ≥ 4 log_2 with over 13% having HI titre ≥ 7 log_2.

The turkeys sampled had a mean ND HI antibodies titre of 5.83 ± 1.29 log_2 with a prevalence of 66.7 %. However, all turkeys having ND antibodies had HI titre of ≥4 log_2 with 58.3 % having HI titre of ≥7 log_2. The mean adult and poults HI titre was 6.57 ± 1.74 log_2 and 4.80 ± 2.00 log_2, respectively

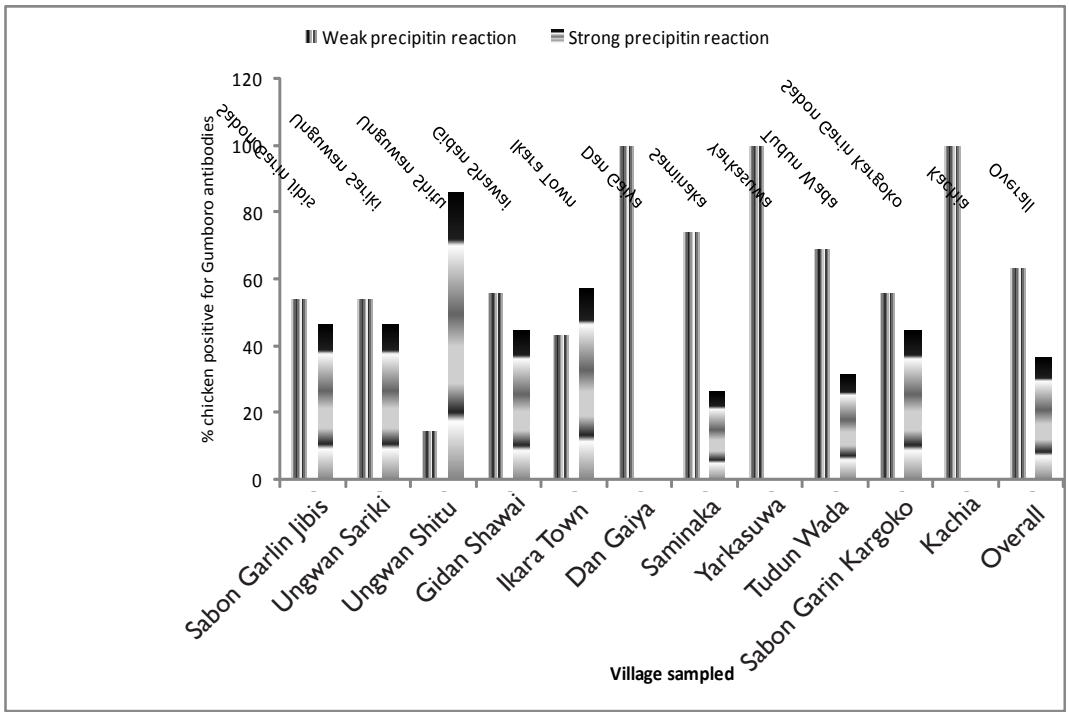


Figure 1: Variation in the strenght of Gumboro precipitin line of local chicken from various villagesin Kaduna State, Nigeria.

Gumboro disease antibodies

All sampled villages had chickens with IBD antibodies though only 55.3 % of chickens were sero-positive with 66 % of positive chickens having a strong precipitin line on AGID test. Though not statistically

significant ($p > 0.05$), young chickens sera produce stronger precipitin line than adults.

Chickens flocks with IBD antibodies had ND HI titre < 4 log2 ($p = 0.042$) with 75.4 % IBD sero-positive adult chickens having ND antibodies HI titre of < 4 log2 ($Chi^2 = 7.62$; $p = 0.006$). Similarly, 78.6% IBD sero-positive cocks had ND antibodies HI titre < 4 log2 ($Chi_2 = 11.49$; $p = 0.001$). In Unguwan Sarki, 85.7 % of chickens without IBD antibodies had ND HI titre ≥ 4 log2 ($p = 0.013$). All turkeys, ducks and geese sampled were negative for IBD antibodies.

Discussion

Poisoning was identified by the study as cause of local chicken loss either as incidental poisoning or as pest in farmlands by scavenging on planted insecticide treated seedlings. The panic sales of sick poultry result in spread of the infection as most farmers restock their flock through purchases from local markets (Assam et al., 2010).

The study confirms previous reports prevalence of fowl pox as a constraint in local poultry production. Clinical pox observed during the study might be due to lack of vaccination of the poultry with the more severe form in young chickens causing ocular and oral lesions leading to starvation and death. Duck viral enteritis or hepatitis are probable causes of blindness in ducklings and enlarged livers reported by farmers.

The study further highlights previous suspicion indicating active circulation of influenza A viruses among LP in some LGAs in Kaduna state of Nigeria (Durosinlorun, 2008). The detection of influenza *A antigen* from ducklings confirms reports of their susceptibility to influenza virus infection (Hinshaw *et al.,* 1980). However, it also highlights the role of domestic ducks in the maintenance of influenza virus in the environment (Webster and Hulse, 2004). A decrease level of virus shedding probably due to increased level of population immunity might reduce chances of Influenza A antigen detection in this study despite prior report of local poultry exposure to H5N2 (Terregino *et al*., 2007; Durosinlorun, 2008).

The limitation of this study was the inability to determine the viral subtype because the positive specimen was exhausted. However, the influenza A virus detected in this study is likely a low pathogenic H5 subtype as the poultry were apparently healthy and H5N2 antibodies have been reported in chickens in the sampled villages (Durosinlorin, 2008). The potential of LPAI becoming HPAI after circulating in poultry justifies the need for continuous surveillance as establishment of HPAI in local poultry would be difficult and expensive to eradicate since they constitute over 80 % of national poultry and are widely dispersed with minimal biosecurity (Villarreal and Flores, 1997). They are also in close contact with the wild birds at the wildlife-livestock interface.

The inability to demonstrate AIV in embryonated eggs might be due to the failure of the duck isolate to haemagglutinate chicken RBC or the chicken isolate can grow in chicken embryonated eggs without causing embryo death (Medeiros *et al.,* 2001). However, it might also be as a result of poor quality of the specimen and storage temperature during transportation leading to absence of viable virus in the specimen (Terregino *et al.*, 2007).

Contrary to previous reports, all LP sampled during this study were negative for antibodies against influenza type A (Durosinlorin, 2008). The discrepancy might be as a result of weaning of antibodies over a two year period between the studies; different type of antigen used or a difference in the serologic test used as the previous study screened the specimens using a more sensitive HI test unlike the AGID used in this study (Durosinlorin, 2008; Meulemans *et al.,* 1987). Furthermore, the ability of AGID test to detect mainly immunoglobulin M which usually lasts for at most six months raises

questions of its use as a screening test for LP which live longer than exotic poultry and are not regularly tested for AI as in developed countries (Swayne *et al.,* 1998). Moreover, the antigen used in the AGID test might have been in high concentration compared with antibody in the test sera resulting in less than optimal proportions of antigen and antibody mix hence preventing formation of precipitin line between antibody and antigen wells (Amos, 1981).

The study confirmed earlier reports that ND is endemic in local chickens though the overall sero-positivity is lower than previous reports in Kaduna State of ND in local chickens (Ezeokoli *et al.,* 1984; Nwanta *et al.,* 2006) but similar to the prevalence in Plateau State (Adu *et al.,* 1986). Since the LP were not vaccinated, the ND antibody might have been derived from field virus challenge or vaccinal virus ingestion from poultry faeces spread on farms as manure and vaccinated exotic chickens kept with LP. The absence of antibodies in chickens in Dan Gaiya village might be due to ongoing challenge whereby ND antibodies were neutralized by field virus or as a result of immune suppression caused by IBD infection. However, the low ND antibodies HI titre of local chickens might not confer for protection resulting in an epidemic when challenged by a virulent field ND strain which could be misdiagnosed as HPAI. This would result in depopulation of LP within the community with consequent loss of a rare gene pool, community's source of protein and livelihood.

The low population of chickens with ND antibody titre ≥ 4 $\log_2$, though similar to findings in Bauchi and Gombe States in Nigeria (Waziri, 2009), implies ND virus spread among the chicken population would be extensive (Boven et al., 2008). However, the study findings revealed an increase in the chickens population with protective ND antibodies HI titre compared with previous studies (Nwanta *et al.,* 2006; Musa, 2008). Similar to a Ugandan report, this study indicates that two-thirds of the local chicken population in Kaduna State are susceptible to ND virus infection (Mukiibi-Muka and Olaho-Mukani, 1998).

The study findings revealed circulation of ND virus in Kachia due to the high antibody titre of chickens with more than half of the sero-positive chickens with antibody titre ≥ 7 $\log_2$ (Gutierrez-Ruiz *et al.,* 2000). However, only chickens in Kachia and Yar kasuwa would be protected from mortality though none of the villages would be protected from either disease transmission nor did they attained flock immunity (Boven et al., 2008). Though ND is endemic in Nigeria, the low prevalence of ND virus antibodies in sampled villages implies local chickens in Nigerian villages remain highly susceptible to virulent ND virus infection (Gutierrez-Ruiz *et al.,* 2000). This study revealed that adult chickens are less susceptible to ND virus infection than growers possibly due to multiple exposures to field virus of adults or severe immune depression in growers as a result of concurrent infection with IBD virus.

Though turkeys were reported to respond poorly to vaccination with La Sota strain (Alexander, 1997), the better *immunogenic* response of turkey in this study compared with chicken and duck is probably because the circulating strain of ND virus is better adapted to turkey. However, ducks described as refractory to ND, had a higher mean HI titre than chicken indicating that ducks are susceptible to ND and also more immunogenic to the circulating field ND strain than chickens (Alexander, 1997). The better immunogenic response of turkeys and ducks ensured that most turkey and ducks sampled had protective ND titre and they might act as source of ND infection to chickens.

The prevalence of IBD antibodies in all the villages sampled is in conformity with earlier studies that identify IBD as one of the diseases limiting rural poultry production (Assam, 1997). The antibodies in these local chickens with no history of vaccination are most likely from infection

with field viruses. The infection probably resulted in *immune-depression* hence reduced antibody production as seen in low antibody titre to ND corresponding strong AGID reaction (Lukert and Saif, 1998). The association between presence of IBD antibodies in adult chickens and cocks with corresponding lack of protection against mortality from ND further highlights the immune-depressive effect of IBD. However, the immune depression might increase the susceptibility of these chickens to common infections thereby reducing production and increasing mortality (Lukert and Saif, 1998). The strong AGID reaction of over two–thirds of sampled chickens indicates a recent challenge resulting in increased precipitin antibody production. The AGID reaction is stronger in growers than adults because growers have a functional bursa unlike adult chickens (Lukert and Saif, 1998).

The study demonstrates that Influenza A virus is circulating among apparently healthy local ducks in Dan Gaiya village. Outbreak of ND in local chickens in Kaduna State could be misdiagnosed as HPAI since local chickens are highly susceptible to ND. Local poultry are also exposed to continuous challenge from fowl pox and IBD viruses and are not protected. There is need for continuous active AI surveillance in LP. Newcastle disease, IBD and FP control in LP should be integrated in HPAI control program while a study should be conducted to investigate the sensitivity of AGID test as a screening test for AI in local poultry.

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References

Abdu P A, Mera U M and Sa'idu L 1992: A study on chicken mortality in Zaria, Nigeria. World's Poultry Congress, Amsterdam, The Netherlands, 20th – 24th September, 1992.

Adu F D, Edo U and Sokale B 1986. Newcastle disease: the *immunological* status of Nigerian local chicken. *Tropical Veterinarian*, 4: 149-152.

Alexander D J 1997. Newcastle disease and other *avian paramyxoviridae* infections. In: B. W. Calnek, H. J. Barnes, C.W. Beard, L. R. McDougald and Y. M. Saif (eds.). Diseases of Poultry. 10th ed. *Iowa University Press.* Ames, Iowa, USA, Pp. 541 – 569.

Allan W H and Gough R E A 1974. Standard haemagglutination *inhibition* test for Newcastle disease. I. A comparison of macro and *micro* methods. *Veterinary Records,* 95(6): 120 – 123.

Amos W M G 1981. *Immunologic* techniques. In: Basic *Immunology*. Butterworths and Co. (Publishers) Ltd., Pp.71 – 72.

Assam A 1997. Disease control in local poultry kept under different management systems in Zaria. DVM *Research project, Ahmadu Bello University,* Zaria, Pp. 23- 45.

Avian Influenza Control Programme (AICP) (2007). The Integrated National *Avian Influenza* and Pandemic Response Plan.

Boven M V, Bouma A, Fabri T H F, Katsma E, Hartog L and Koch G 2008. Herd immunity to Newcastle disease virus in poultry by vaccination. *Avian Pathology*, 37(1): 1 – 5.

Durosinlorun A 2008. *Avian influenza* (H5N2) antibodies in local chicken and awareness level of highly *pathogenic avian influenza* in Kaduna State. MSc. *Thesis, Ahmadu Bello University,* Zaria – Nigeria, Pp. 1-91.

Easterday B C, Hinshaw V S and Halvorson, D.A 1997. *Influenza*. In: Diseases of Poultry. 10th Ed. Calnek B.N., Barnes, H.J., Beard, C.W., McDougald, L.R. and Saif, Y.M. Ed. *Iowa State University Press,* Ames, Iowa, USA, Pp. 583-608.

Ezeokoli C D, Umoh J U, Adesiyun A A and Abdu,

P A 1984. Prevalence of Newcastle disease virus *antibodies* in local and exotic chicken under different management systems in Nigeria. *Bulletin of Animal Health and Production in Africa,* 2 (3): 253-257.

Gutierrez-Ruiz E J, Ramirez-Cruz G T, Gamboa E I C, Alexander D J and Gough, R E 2000. A *serological* survey for avian infectious *bronchitis virus* and Newcastle disease virus antibodies in backyard (free-range) village chickens in Mexico. *Tropical Animal Health and Production*, 32:381-390.

Harai K, Shimakura S and Hirose M 1972. *Immunodiffusion* reaction of *avian infectious bursal virus. Avian Diseases*, 16:961 – 964.

Hinshaw V S, Webster R G and Turner B 1980: The perpetuation of *orthomyxoviruses* and *paramyxoviruses* in Canadian waterfowl. *Canadian Journal of Microbiology*, 26: 622-629.

Lukert P D and Saif Y M (1998). *Infectious bursal* disease infection. In: B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald and Y. M. Saif (eds.). Diseases of Poultry. 10th ed. *Iowa University Press. Ames*, Iowa, USA, Pp. 721 – 738.

Medeiros R, Eseriou N, Naffakh N, Naffakh N, Manugurra J C and Van der Werf S 2001. *Haemagglutinn* residues of recent human A (H3N2) *influenza* viruses that contribute to inability to agglutinate chicken erythrocytes. *Virology*, 289: 74-85.

Meulemans G, Carlier M C, Gonze M and Petit P 1987. Comparison of *hemagglutination* – inhibition test, agar gel precipitin and enzyme – linked immunosorbent assay for measuring antibodies against influenza viruses in chickens. *Avian Diseases,* 31: 560 – 563.

Mukiibi-Muka G and Olaho-Mukani W 1998. *Serological* survey of antibodies to infectious *bronchitis* virus, Newcastle disease virus and mycoplasma in commercial and indigenous chicken in Uganda. Proceedings of the International *Symposium* on Infectious *Bronchitis* and *Pneumovirus* Infections in Poultry. (Rauischholzhausen, 1998, World *Veterinary Poultry Association and Institut fur* Geflugelkrankheiten Justus-Liebig-Universitat, Giessen, Germany), Pp.157 – 165.

Musa U 2008. Vaccination and comparison of efficacy of Newcastle disease vaccine strains I2 and La Sota in village chickens in Plateau State. PhD Thesis. Department of Veterinary Surgery and Medicine, *Ahmadu Bello University,* Zaria, Nigeria, Pp. 1-199.

Nicolas G, Giovanni C, Saliha H, Scott, H N, Ward H, John Y T, Julien C, Tim D, Tony J, Patrici G, Isabella M, Alice F, Capua I, Manu S, Micheloni P, Ottosson U, Mshelbwala J H, Lubroth J, Domenech J and Monicat J 2008: Evidence of infection by H5N2 highly *pathogenic avian influenza* viruses in healthy wild waterfowl. PLoS *Pathology*, 4(8): e1000127.

Nwanta J A, Umoh J U, Abdu P A, Ajogi I and Alli – Balogum J K 2006. Management of losses and Newcastle disease in rural poultry in Kaduna State, Nigeria. *Journal of Animal Production,* 33(2): 274 – 285.

Nwosu C C 1997. Is the local fowl essential or non essential? A paper presented at poultry workshop National Agricultural and Extension Rresearch and Liaison Services, A.B.U., Zaria, December.

RIM 1993. Nigerian Livestock Reserve Resource Inventory & Management Report, Vol. 1-4. FDLPCS.

Swayne D E, Senne D A and Beard C W 1998. *Avian influenza*. In: D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson and W. M. Reed (eds.). A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 4th ed. American Association of Avian *Pathologist*, Kennett Square, PA, Pp. 150 – 155.

Swayne D E and Jackwood M P 2008. *Pathobiology* of *avian influenza* virus infections in birds and mammals. In David. E. Swayne (ed.): *Avian Influenza* 1st ed. Blackwell: Ames, Iowa – USA, Pp. 87-122.

Terregino C, De Nardi R, Gugerti V, Scremin M, Raffini E, Martin A M, Cattoli G, Bonfanti L and Capua I 2007. Active surveillance for *avian influenza* viruses in wild birds and backyard flocks in Northern Italy during 2004 – 2006.

Avian Pathology, 36 (4): 337 – 344.

Villarreal C L and Flores A O 1997. The Mexican *avian influenza* H5N2 outbreak. In D.E. Swayne & R.D. Slemons (Eds), Proceedings of the Fourth international symposium on *Avian Influenza* (pp. 18 – 22). *US Animal Health Association*, Georgia Center for Continuing Education, The University of Georgia, Athens, GA, USA.

Waziri M I 2009. Sero-diagnosis and survey of avian influenza and Newcastle disease (using rapid antigen test kit) in poultry in Bauchi and Gombe States, Nigeria. MSc. Thesis, Ahmadu Bello University, Zaria – Nigeria, Pp. 1-100.

Webster R G and Hulse D J 2004. *Microbial* adaptation and change: avian *influenza*. Revue Scientifique et Technique, 23 (2): 453 – 465.

World Organization for Animal Health (OIE) 2009. Chapter 2.7.12 *Avian Influenza.* In: Terrestrial *Animal Health Code*, sixteenth ed., OIE, Paris, France, Pp. 279 – 285.

HAEMO AND ECTO-PARASITES OF CATTLE IN THE TRANS-BOUNDARY AREAS OF OGUN STATE, NIGERIA

HEMOPARASITES ET ECTOPARASITES DE BOVINS DANS LES REGIONS TRANSFRONTALIERES DE L'ETAT D'OGUN AU NIGERIA

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Abstract

An assessment of the parasites of cattle entering Nigeria from the Republic of Benin by hoof along a major trans-boundary route was carried out. Of the 245 blood samples analysed, 50.20% were positive for blood protozoan *Babesia sp.* (20.41%), *Trypanosoma sp.* (11.84%), *Anaplasma spp.* (5.71%), mixed *Anaplasma spp.* and *Babesia spp.* (3.67%) and mixed *Babesia spp.* and T*rypanosoma spp.* (8.57%). All the animals sampled had varying degrees of tick infestation. The 3756 ticks collected were *Rhipicephalus appendiculatus* (28.75%), *Amblyomma variegatum* (24.41%), *Boophilus decoloratus* (19.68%), *Boophilus microplus* (12.97%), *Amblyomma hebraeum* (12.59%), *Hyalomma spp.* (1.09%) and Amblyomma gemma (0.51%). Other parasites found included *Haematopinus sp*. (6.53%); *Demodex bovis* (7.35%) and *Dermatophilus congolensis* (6.12%). The very high parasitic load observed in the animal population in this study is an indication that many parasites enter the country on daily basis via animate vectors. These animals may be carriers of a wide range of economically important diseases, the control of which may be very expensive. Since the potential for increasing livestock production in Nigeria can only be fully realized if the animals are adequately protected against major diseases, the animals in border areas should be regularly monitored and screened for diseases of economic and/ or *epizootic* importance to minimize spread of trans-boundary diseases to the national herd.

Keywords: *Haemo-parasites, Ecto-parasites*, Cattle, Trans-boundary, Nigeria

Résumé

Une étude a été menée sur les parasites des bovins en provenance de la République du Bénin entrant au Nigéria par la principale voie transfrontalière. Sur les 245 échantillons de sang analysés pour la détection des protozoaires du sang, 50,20% ont été positifs pour les protozoaires suivants : (Babesia spp. (20,41%), Trypanosoma spp. (11,84%), Anaplasma spp. (5,71%). Certains échantillons, par ailleurs, sont des porteurs d'espèces mixtes d'Anaplasma spp. (5,71%) et de Babesia spp. (3,67%) et d'espèces mixtes de Babesia spp. et de Trypanosoma spp. (8,57%).

Tous les animaux sélectionnés présentaient des degrés divers, d'infestation des tiques. Un total de 3756 tiques ont été prélevées de leurs corps au nombre desquelles le Rhipicephalus appendiculatus (28,75%), l'Amblyomma variegatum (24,41%), le Boophilus decoloratus (19,68%), le Boophilus microplus (12,97%), l'Amblyomma hebraeum (12,59%), le Hyalomma spp. (1,09%) et l'Amblyomma

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gemma (0,51%). Parmi les autres parasites détectés figurent le Haematopinus sp. (6,53%), le Demodex bovis (7,35%) et le Dermatophilus congolensis (6,12%).
La très forte charge parasitaire observée dans la population animale faisant l'objet de la présente étude tend à confirmer que de nombreux parasites entrent chaque jour dans le pays par des vecteurs animés. Ces animaux sont potentiellement porteurs de toute une série de maladies fortement préjudiciables à l'économie, dont le contrôle peut s'avérer très coûteux. Partant du fait que la réalisation du potentiel d'accroissement de la production animalière au Nigéria est fortement tributaire de la mise en place d'un système efficace de protection du bétail contre les principales maladies, les animaux des zones frontalières devraient faire l'objet d'un suivi vétérinaire et d'un dépistage régulier des maladies et/ou des épizooties aux retombées dévastatrices pour l'économie en vue de réduire au strict minimum la propagation des maladies transfrontalières et d'éviter la contamination du cheptel national par ces dernières.

Mots clés: Hémoparasites, ectoparasites, bovins/cheptel, transfrontalier, Nigeria

Introduction

The recent incidences of emerging and re-emerging transboundary animal diseases led to very heavy losses all over the world. The outbreaks are associated with huge economic and social impact in many countries (Belak, 2006). The most important diseases are classified by the Office International des Épizooties (OIE) under List A. List A diseases are defined as "transmissible diseases which have a potential for very serious and rapid spread, irrespective of national borders, which are of serious socioeconomic or public health consequence and which are of major importance in the international trade of animals and animal products." These include Foot and mouth disease,Vesicular stomatitis, Swine vesicular disease, *Rinderpest, Peste des petits ruminants*, Contagious bovine pleuropneumonia, Lumpy skin disease, Rift Valley fever, *Bluetongue*, Sheep pox and goat pox, African horse sickness, African swine fever, Classical swine fever, Highly pathogenic avian influenza and Newcastle disease (FAO, 2008).

In sub-Saharan Africa, ticks, tick-borne infections (TBIs), such as *anaplasmosis*, *babesiosis*, cowdriosis and/or tick-associated diseases, such as *dermatophilosis*, together with tsetse-transmitted *trypanosomosis*, constitute arguably the major *pathological* parasite complex responsible for limiting animal production. The presence of such diseases also constrains the introduction of exotic upgraded cattle with a view to improving the approximately livestock industry. A recent report estimated annual world-wide losses associated with ticks and tick-borne diseases to be in the range of 18 billion US dollars. In Africa, losses due to tsetse-transmitted *trypanosomosis* are evaluated between 6 and 12 billion US dollars per year in the cattle industry alone (Matioli *et al.,* 2000). Increasing worldwide movement of commodities, animals and people is also responsible for the globalization of pathogens (De Deken *et al.*, 2007).

A significant amount of cattle enter Nigeria on daily basis by hoof along the trans-boundary areas. Most studies concerning parasites of cattle in Nigeria rarely consider those animals entering the country along the trans-boundary areas.
This study is aimed at identifying the various parasites of cattle entering Nigeria along a major trans-boundary route.

Materials and Methods

Location of study

The study was carried out in Yewa division of Ogun State, Nigeria between February 2006 and July 2007. The location of study is bound to the west by the Republic of Benin, with which it shares 155 kilometres

of International boundary, within latitude 60 151 N and 70 581 N in a deciduous/derived Savannah zone of Nigeria. A total of 245 randomly selected animals from 62 herds of between 56 and 98 heads of cattle entering Nigeria from neighbouring West African Countries by hoof along the Iwoye – Imeko – Olodo – Abeokuta route were observed for various *haemo*- and *ecto*-parasites.

Sample Collection

Samples were collected with the permission of the owners and care was taken to minimise discomfort for the animals (Macaluso *et al*., 2003). About 5ml of blood was collected from the jugular vein of each of 245 randomly selected animals into sterile bijou bottles containing 250µl of 200 mM disodium ethylene diamine tetra acetic acid (Na_2EDTA) as anticoagulant. Animals infested with ectoparasites were noted and the parasites on each of the animals were collected into universal bottles containing 1% formaldehyde. The pus expressed from skin nodules on 18 animals and skin scabs from 15 animals were also collected.

Laboratory examination of the samples

Laboratory examination of the samples was done at the College of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria. The blood samples were screened for the presence of trypanosomes using the buffy coat centrifugation method. Presence of other blood parasites was demonstrated by examination of Giemsa-stained thin blood smear (Soulsby, 1982). Ticks collected from the infested animals were separated and identified using MAFF (1977) identification keys. A drop of collected pus was mixed with 10% *Potassium hydroxide* on a *microscope* slide, a cover slip placed and examined under x40 objective of a binocular microscope (Olympus, Germany) for the presence of Demodex spp. The skin scabs were culturally examined for the presence of Dermatophilus spp. according to the method described by Abu-Samra and Walton, (1977).

Results

The parasites infesting cattle in the transboundary area of Ogun State, Nigeria are presented in Table 1. Of the 245 blood samples analysed, 123 (50.20%) were positive for blood *protozoan*. This include Babesia sp. (20.41%), *Trypanosoma sp*. (11.84%), *Anaplasma sp.* (5.71%), mixed infection of *Trypanosoma sp.* and Babesia sp. (8.57%) and mixed infection of *Babesia sp.* and *Anaplasma sp.* (3.67%).

The species of ticks collected in the course of this study are presented in Table 2. All the animals sampled had varying degrees of tick infestation ranging from a total count of 26 to 280 ticks per

Table 1: Parasites infesting cattle in the transboundary area of Ogun State, Nigeria

Parasite	No of infested animal	%
Babesia alone	50	20.41
Trypanosomes alone	29	11.84
Anaplasma alone	14	5.71
Mixed Trypanosomes and Babesia	21	8.57
Mixed Babesia and Anaplasma	9	3.67
Haematopinus	16	6.53
Demodex	18	7.35
Dermatophilus congolensis	15	6.12

Table 2: Ticks infesting cattle in the transboundary area of Ogun State, Nigeria

Tick	No collected	%
Rhipicephalus appendiculatus	1080	28.75
Amblyomma variegatum	917	24.41
Boophilus decoloratus	739	19.68
Boophilus microplus	487	12.97
Amblyomma hebraeum	473	12.59
Hyalomma spp.	41	1.09
Amblyomma gemma	19	0.51
Total	3756	

animals. Of the 3756 ticks collected, 1080 (28.75%) were *Rhipicephalus appendiculatus,* 917 (24.41%) *Amblyomma variegatum,* 739 (19.68%) *Boophilus decoloratus,* 487 (12.97%) *Boophilus microplus,* 473 (12.59%) *Amblyomma hebraeum*, 41 (1.09%) *Hyalomma spp.* and 19 (0.51%) *Amblyomma gemma. Haematopinus* was the only louse specie identified in this study. They were found on the upper eyelid, inner commissure of the *pinna* and tip of the tail in 16 animals. *Demodex bovis* was seen in all the 18 skin nodules, while *Dermatophilus congolensis* was isolated from all 15 skin scabs collected.

Discussion

The results of this investigation revealed that 50.20% of the cattle entering Nigeria along the transboundary routes habour blood protozoan and all the animals sampled had varying degrees of tick infestation. The widespread presence of ticks on all animals in this study suggested that they could be playing a greater role in disease transmission. Tick borne diseases especially *theileriosis, babesiosis*, *dermatophilosis* and *cowdriosis* seriously limit livestock production and improvement in much of Africa. Also, presence of ticks inhibits animals ability to develop immunity and recover from dermatophilosis (Koney *et al.*, 1996). A survey of *Boophilus sp*. on cattle, sheep and goats in Nigeria between January and December 1973 revealed three species on cattle and of these, *Boophilus decoloratus* was predominant, the others were *B. geigyi* and *B. annulatus* (Dipeolu, 1974). Over a period of one year (January to December 1983), Ugochukwu and Apeh (1985) reported common ectoparasites of small ruminants in selected farms in Nsukka, Nigeria. The species identified in goats include *Ctenocephalides canis, Boophilus decoloratus* and *Amblyomma variegatum*, whilst those of sheep include *C. canis* and *Damalina ovis*. Out of the total of 148 sheep and goats examined for ectoparasites, 68 (45.95%) were infested (Ugochukwu and Apeh, 1985).

In this study, of the 3756 ticks collected, 28.75% were *Rhipicephalus appendiculatus,* 24.41% *Amblyomma variegatum,* 19.68% were *Boophilus decoloratus*, 12.97% *Boophilus microplus* and 12.59% *Amblyomma hebraeum. Rhipicephalus appendiculatus*, the brown ear tick, is a three host tick but adults and immature feed on the same hosts which include cattle, other livestock and antelope. It is the major vector of the Theileria parva group of diseases, Nairobi sheep disease virus, and is also a vector of Theileria taurotragi, Ehrlichia bovis, Rickettsia conori and Thogoto virus (Kahn, 2005). Heavy infestations on susceptible Bos taurus cattle sometimes cause fatal toxaemia, loss of resistance to various infections and severe damage to the host's ears. *Boophilus annulatus* on the other hand is a one host tick. It develops rapidly in 3 to 4 weeks and results in a heavy tick burden (Kahn, 2005). *Boophilus annulatus* is a

major vector of *Babesiosis* and *Anaplasmosis* (Radostits *et al.,* 2007). *Amblyomma variegatum* is a large, brightly ornamented three host tick. The long mouthparts make it especially difficult to remove manually and frequently cause serious wounds that may become secondarily infected by bacteria or screw-worms (Kahn, 2005). It is a vector of *Cowdria ruminatum*, the *rickettsial* agent of Heartwater and also transmits *Dermatophilus congolensis* and *Theileriosis* (Radostits *et al.,* 2007).

Primary factors in the extensive distribution and prevalence of many tick species and tick-borne disease agents are movement of tick-infested livestock over great distances (Kahn, 2005). Complete eradication of these ticks is extremely difficult because of the persistence of the ticks, especially multi-host ticks on wild animals and the ability of adult ticks to live for very long periods away from a host. On the other hand, continuous treatment to restrain the tick population is highly conducive to the development of resistance (Radostits *et al.,* 2007). Since the control of ticks by acaricides is the most common method of tick control world-wide (Kahn, 2005), strategic use of these acaricides during the period of high tick burden based on the life-cycle and epidemiology of the ticks is recommended.

Dermatophilus congolensis was isolated from skin scabs. *Dermatophilosis* caused by the bacterium *Dermatophilus congolensis* is a major constrain to attempts at increasing meat and milk production in Africa because imported exotic breeds needed to improve productivity are particularly susceptible (Morrow and Koney, 1996).

Under natural conditions, many animal species including cattle, sheep, goat, equines, camels and humans are susceptible to *D. congolensis* (Van Tunder and Horner, 1994). A severe *chronic* form of dermatophilosis is associated with tick infestation particularly *Amblyomma variegatum*. The importance of *Amblyomma variegatum* ticks in aggravating clinical *dermatophilosis* in different livestock species has been demonstrated by Morrow and Koney (1996). The successful use of more productive cattle in Africa and Nigeria in particular will depend on effective control of *dermatophilosis*. The only effective control measure at present is the use of acaricides to reduce tick numbers but the long term use and misuse of acaricides may lead to the development of acaricides residues in animal products and environmental pollution. Preventive measures for dermatophilosis should aim to interrupt the chain of factors required for the development and occurrence of the disease. In this regard, animals with skin lesions due to dermatophilosis have to be separated from non-infected ones or be culled.

The very high parasitic load observed in the animal population in this study is an indication that many parasites enter the country on daily basis via animate vectors. These animals may be carriers of a wide range of economically important diseases, the control of which may be very expensive. This may also result in additional cost of production to the owners of in-contact animals. Since the potential for increasing livestock production in Nigeria can only be fully realized if the animals are adequately protected against major diseases, the animals in border areas should be regularly monitored and screened for diseases of economic and/ or epizootic importance to minimize spread of trans-boundary diseases to the national herd.

In conclusion, there is need for a nationally coordinated comprehensive study of the parasite-host interaction, life-cycle and epidemiology of ectoparasites. Also, there is need to adopt the policy of removing parasites of trade cattle before transportation to consumption areas.

Impact

An assessment of the parasites of cattle entering Nigeria from the Republic

of Benin by hoof along a major trans-boundary route indicated that 50.20% of the animals habour blood protozoan and all the animals sampled had varying degrees of tick infestation. The widespread presence of ticks on all animals in this study suggested that they could be playing a greater role in disease transmission.

References

Abu-Samra M T and Walton G S. 1977. Modified techniques for the isolation of Dermatophilus spp. from infected material. Sabouraudia, 15: 23-27.

Belak S. 2006. Molecular diagnosis of viral diseases, present trends and future aspects: A view from the OIE collaborating centre for the application of PCR methods for diagnosis of viral diseases in Veterinary Medicine. Vaccine, 25: 5444-5452.

De Deken R, Martin V, Saido A, Madder M, Brandt J and Geysen D. 2007. An outbreak of East Coast Fever on the Comoros: A consequence of the import of immunized cattle from Tanzania *Veterinary Parasitology,* 143: 245-253.

FAO, 2008. Manual on Livestock Disease Surveillance and Information Systems. Visited March 18, 2009 from http://www.oie.int/eng/maladies/en_classification.htm

Kahn C M. 2005. The Merck Veterinary Manual, 9th edition, Merck & Co., Inc. Rahway, U.S.A.

Koney E B M, Morrow A N, Heron L A and Ambrose NC, 1996. Studies on the epidemiology of dermatophilosis in Ghanaian cattle. In Proceeding of 2nd International conference on tick-borne pathogens at the Host-vector interface, Kruger National park, South Africa, August 1995.

Macaluso K R, Davis J, Alam U, Korman A, Rutherford JS, Rosenberg R and Azad AF, 2003. Spotted fever group Rickettsiae in ticks from the Masai Mara region of Kenya. *American Journal of Tropical Medicine and Hygiene.* 68: 551-553.

MAFF, 1977. Technical Bulletin No. 18. Manual of Veterinary Parasitological Laboratory Techniques. 2nd Edition. Her Majesty Stationery Office. London, pp: 99.

Mattioli R C, Pandey V S, Murray M and Fitzpatrick J L, 2000. Immunogenetic influences on tick resistance in African cattle with particular reference to *trypanotolerant* N'Dama (Bos taurus) and *trypanosusceptible* Gobra zebu (Bos indicus) cattle. Acta Tropica. 75: 263-277.

Morrow A N and Koney E B M, 1996. Control of Amblyomma variegatum and dermatophilosis on local and exotic breeds of cattle in Ghana. In "Proceedings of a workshop on the control of *Amblyomma variegatum* ticks and associated diseases". Accra, Ghana. November 1994.

Radostits O M, Gay C C, Hinchcliff K W and Constable P D. 2007. Veterinary Medicine: A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th ed., Saunders Elsevier, Spain. pp: 1599-1602.

Soulsby E J L. 1982. Helminths, Arthropods and Protozoa of domestic animals. 7th edition. Published by the English Language Book Society and Bailliere Tindall. London, pp: 516-538.

Ugochukwu E I and Apeh A O, 1985. Prevalence of Ectoparasites of small ruminants in Nsukka, Nigeria. International Journal of Zoonoses, 12: 313-317.

Van Tunder E M and Horner R F, 1994. Dermatophilosis. In "Infectious diseases of livestock". Edited by Coetzer, Thompson and Tustin. Oxford University Press, Cape Town, RSA. pp: 2825-2852.

Prevalence of tick-borne infections in extensive cattle management system in West Pokot District, Kenya.

Prévalence des infections transmises par les tiques dans le système de gestion extensive du bétail dans le district de West Pokot, au Kenya.

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Abstract

A study was conducted in West Pokot District, Kenya to obtain baseline data on the epidemiology of tick-borne diseases in cattle under extensive management. A total of 197 serum samples were analyzed for antibodies against *T. parva, A. marginale,* and *B. bigemina*. Blood smears were examined for *hemo-parasites* and body tick counts were conducted for relative abundance. Overall sero-prevalence was 25.8 %, 53.2 % 53.7 % for *T. parva, A. marginale* and *B. bigemina,* respectively. Prevalence of antibodies to *A. marginale* and B. bigemina was significantly higher in animals in pastoral areas than those in agro-pastoral areas while antibodies to *T. parva* were higher in agro-pastoral than in pastoral areas. Animals grazing on private paddocks had significantly lower prevalence of antibodies against *A. marginale* and *B. bigemina* than those grazing on communal land ($p < 0.05$). Animal age, breed and grazing management were significantly associated with *T. parva* sero-prevalence while grazing and veterinary service providers were significantly associated with *A. marginale sero*-prevalence. Age and production system were associated with *B. bigemina sero*-prevalence. Out of 176 blood smears, 37.6% were positive for *Theileria piroplasms* while 5.8% and only 0.6% were positive for Anaplasma and *Babesia piroplasms,* respectively. The most prevalent tick species was *R. appendiculatus, B. decoloratus, R. evertsi, Amblyoma spp, Hyalomma spp*, in that order. The findings indicate that tick-borne diseases and their vectors are prevalent and could be serious constraints to cattle production in the study area. Current efforts to treat affected animals are largely ineffective and there is a need to advise livestock keepers on their implications and control. The study provides a basis for detailed epidemiologic studies to provide further evidence on incidence and prevalence of tick-borne diseases and guidelines useful to extension workers in addressing the needs of the livestock keepers.

Key words: *epidemiology*, sero-prevalence, risk factors, ticks, Tick-borne diseases, grazing management, arid and semi-arid lands.

Résumé

Une étude visant à obtenir des éléments d'information bien précis sur l'épidémiologie des maladies transmises par les tiques chez les bovins en élevage extensif a été menée dans le district de West Pokot, au Kenya. Un total de 197 échantillons de sérum ont été analysés pour les anticorps contre les infections à T. parva, A. marginale, et B. bigemina. Les frottis sanguins ont été examinés aux fins de détection des hémoparasites, et le décompte des tiques a permis de se rendre compte de leur abondance relative. Le taux global de séroprévalence était de 25,8%, 53,2% 53,7% pour les infections à T. parva, A. marginale et B. bigemina respectivement. La prévalence des anticorps contre les infections à A. marginale et B. bigemina a été nettement plus élevée chez les animaux des zones pastorales que chez ceux des zones agro-pastorales contrairement à la prévalence des anticorps

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contre les infections à T. parva qui était plus élevée dans le secteur agro-pastoral que dans les zones pastorales. Les animaux qui pâturent sur des parcelles privées présentaient une prévalence nettement plus faible d'anticorps contre les infections à A. marginale et B. bigemina que ceux qui utilisent les terres communales comme aires de pâturages ($p < 0,05$). Il existe une corrélation très étroite entre l'âge, la race et la gestion des pâturages des animaux et la séroprévalence de T. parva, tout comme il existe un lien évident entre le système de pâturage, les fournisseurs de services vétérinaires, d'une part, et de l'autre, la séroprévalence des infections à A.marginale. L'âge et le système de production animale ont été associés à la séroprévalence des infections à B. bigemina. Sur les 176 frottis sanguins analysés, 37,6% échantillons ont été trouvés positifs aux tests de détection de piroplasmes Theileria, contre 5,8% et o,6% seulement aux tests de détection d'Anaplasma et de piroplasmes Babesia, respectivement. Les espèces de tiques les plus répandues étaient R. appendiculatus, B. decoloratus, R. evertsi, Amblyoma spp, Hyalomma spp, dans cet ordre. Les résultats indiquent que les maladies transmises par les tiques et leurs vecteurs sont fréquentes et pourraient créer de sérieux problèmes pour la production du bétail dans la zone étudiée.

Mots clés: Epidémiologie, séroprévalence, facteurs de risque, tiques, maladies transmises par les tiques, gestion des pâturages, terres arides et semi-arides.

Introduction

Arid and semi-arid lands (ASALs) in Kenya account for 50% of the livestock, 3% of agricultural output and 7% of commercial output (Government of Kenya, 2003). Livestock production in these areas is often the only economic activity sustaining the livelihoods of the inhabitants. In spite of this importance, livestock production is at risk from a variety of constraints including a range of animal diseases, poor husbandry practices, inadequate nutrition and lack of markets for livestock and their products (Rubaire-Akiiki *et al.,* 2004). Tick-borne diseases (TBDs) are a major constraint especially in the areas where cattle are kept under the open grazing systems due to the ease of spreading ticks among cattle herds. Among the TBDs, *theileriosis* (East Coast fever), *anaplasmosis, babesiosis* and heartwater (cowdriosis) are the most important (Otim, 2000).

East Coast fever (ECF), the focus of this study, is caused by Theileria parva and transmitted trans-stadially by the brown ear tick *Rhipicephalus appendiculatus*. The disease is the main cause of calf mortality among the East African indigenous cattle, with mortality rates of 40–80% reported among the Maasai pastoralist herds (Di Giulio *et al*. 2003) and thus is a serious threat to livestock production in this system. Out of an estimated 13.5 million cattle in Kenya, 52.8% are found in *R. appendiculatus* infested areas and as much as 80% are thought to be at risk from ECF (Dolan and Young, 1981). Poor understanding of the epidemiology of ECF has resulted in poor disease management practices among pastoralists. There is evidence that the disease is spreading to new areas. For instance, the Pokot have reported it as a relatively new disease which often overshadows major trans-boundary livestock diseases such as contagious bovine pleuropneumonia, foot-and-mouth disease, Rift valley fever, and lumpy skin disease (Ngotho *et al.,* 1999).

A better understanding of vector distribution and prevalence of the disease is crucial in assessing the extent of the disease and its potential impact in a particular area and in planning control strategies (Maloo *et al.,* 2001). The present study seeks to provide an understanding of the transmission dynamics of tick-borne infections in traditional herds in pastoral and agro-pastoral livestock production systems. The specific objective of the study was to obtain baseline data on the epidemiological states of tick-borne infections that could be used in the formulation of extension messages targeting their control in the region.

Materials and Methods

Study area

The study was conducted in West Pokot district between November, 2006 and January, 2007. The district is divided into ten administrative divisions, 58 locations and 188 sub locations. There are three main livelihood zones with pastoral zone supporting 45% of the population, agro-pastoral and mixed farming economy zones supporting 29% and 26%, respectively (ALRMP, 2005).

Selection of study sites

The district was stratified into the upper mid-land (UM) and the lowland zone (L) according to the classification by Jaetzold and Schmidt, (1983). Three administrative divisions, Kacheliba and Kongelai in the lowland and Chepareria in the upper mid-land zone were purposively selected. The study was conducted in 23 geo-referenced villages (eight in Kacheliba Division, three in Kongelai and twelve in Chepareria Division). The villages were selected to ensure the whole division was represented. Most villages were accessible by road except the ones along Kenya-Uganda border and Turkana District that were a security risk.

Data collection

Collection of blood samples

Serum samples were collected from randomly selected animals in herds from areas where a participatory appraisal had been previously conducted. Herd owners were requested to respond to a structured questionnaire to capture information pertaining to their households and herds.
Blood was collected from animals by jugular veni-puncture using vacutainer tubes which were stored in cool boxes with ice while in the field and later left overnight at room temperature for serum separation. After separation serum samples were delivered to the laboratory in ice boxes and stored at -20˚C. Enzyme-linked-immunosorbent assay (ELISA) was used to measure antibodies to *Theileria parva, Anaplasma marginale* and *Babesia bigemina.* The ELISA protocols for the measurement of antibodies for *T. parva* (Morzaria *et al.* (1999), *B. bigemina* (Tebele *et al.* (2000) have been described previously. The results were expressed as percent positivity (PP) according to Wright *et al.* (1993) (PP = optic density of test serum/ optic density of strong positive) x100). For *T. parva,* a sample was considered positive if the percent positivity value was 20 or above; while for *A. marginale and B. bigemina*, the cut-off PP value was 15.

Preparation of blood and Lymph-node smears

Thick and thin blood smears were prepared from blood collected from the marginal ear vein of all the animals that were bled. Lymph node biopsy smears were collected from animals suspected to be sick at the time of sample collection for microscopic examination based on disease symptoms such as deep coughs, animals showing swollen lymph nodes and anorexia. The slides were air-dried and fixed in methanol for 5 minutes and stored in slide boxes. In the laboratory, the slides were stained with 10% *Giemsa* solution for 30 minutes and examined for presence of *Trypanosomes, Theileria, Anaplasma* and *Babesia haemo*-parasites under the oil immersion objective at x100 magnification.

Collection and examination of ticks

Whole body tick counts were carried out on cattle selected for sampling. Representative tick samples were collected by plucking them from cattle using entomological forceps (Horak, 1982). Partially fed ticks were placed in 20 ml universal bottles with cotton wool dampened with sterile water while the engorged ticks were placed in 96% alcohol. The bottles were placed in a cool box maintained at approximately 4˚C by ice packs for dispatch to the laboratory to minimize death of the vector ticks and bacterial spoilage. Once at the laboratory questing ticks were stored at -20^{0}C before processing for identification.

Tick species, sex and state of feeding of female ticks were determined. Live ticks were dissected and salivary glands stained for infection prevalence assessment.

Data Handling and Analyses

Laboratory and field data were transferred to a database, collated and stored in Microsoft Excel. The data were then exported to SPSS system for Windows Version 12.0.1 for descriptive statistical analysis of frequencies, means and median score estimation.

General linear model (GLM) was used to identify risk factors for *T. parva, A. marginale* and *B.bigemina* sero-prevalence by using antibody prevalence as the outcome variable against the potential risk factors (dependent variables). A dichotomous response variable was created from the absolute percentage positive (PP-values) and coded as positive or negative using a fixed cut off of 15 for *A. marginale* and *B. bigemina*, and 20 for *T. parva*. Age, the type of production system, division, breed, grazing management, tick control practices and frequency, acaricide mixing, method of application and veterinary service provider were used as fixed effects. Analyses were conducted using SPSS software version 12.1, SPSS Inc., Chicago, IL, USA).

A univariate logistic regression procedure was used to test the significance of the difference in the *sero*-prevalence between independent categories separately. All variables with univariate likelihood ratio test $p \leq 0.2$ were carried forward to a multivariate analysis. Logistic regression analysis was performed to investigate the association between the sero-prevalence of the tick-borne diseases and the selected independent variables with a logistic link function. The logistic regression models were fitted with all the variables for each disease, separately. All independent variables were included in the robust model. Both the main effects and their positive interaction terms were included in the model but only those satisfying a $p < 0.1$ significance level were retained in the final model. A backward elimination approach was used to judge important variables to be retained in the final model with non-significant main effects removed and previously removed main effects assessed for re-inclusion at each step. To check for multi-colinearity between factors in the final model, bi-variate correlations for factor variables were examined.

Table 1: *Acaricide* application frequency in West Pokot District as determined in the household survey.

Frequency of application	Percentage of respondents (%)
once weekly	17.9
once fortnightly	48.2
once every 3 weeks	1.8
once a month	19.6
others (depending on tick load)	12.5

Results

Description of the farming systems

Results from the household survey indicated that a majority of the households (96.4%) derived their livelihoods from farming. Land tenure was communal in the pastoral divisions while the agro-pastoral divisions had a mixture of communal (75.7%) and private land ownership (24.3%). The mean land size per household in the agro-pastoral divisions was between 5 and15 acres. All the households in pastoral divisions kept Zebu as their main cattle breed while only 16.2% of households in agro-pastoral divisions kept the Zebu and 83.8% kept Zebu and Friesian/Aryshire crossbreds. Within the divisions practicing agro-pastoral production system cattle were grazed in both individual (86.5%) and communal pastures (13.5%). Watering was mainly from streams and rivers for 81.1% of the herds with the remaining 18.9% of the herds getting water from other sources

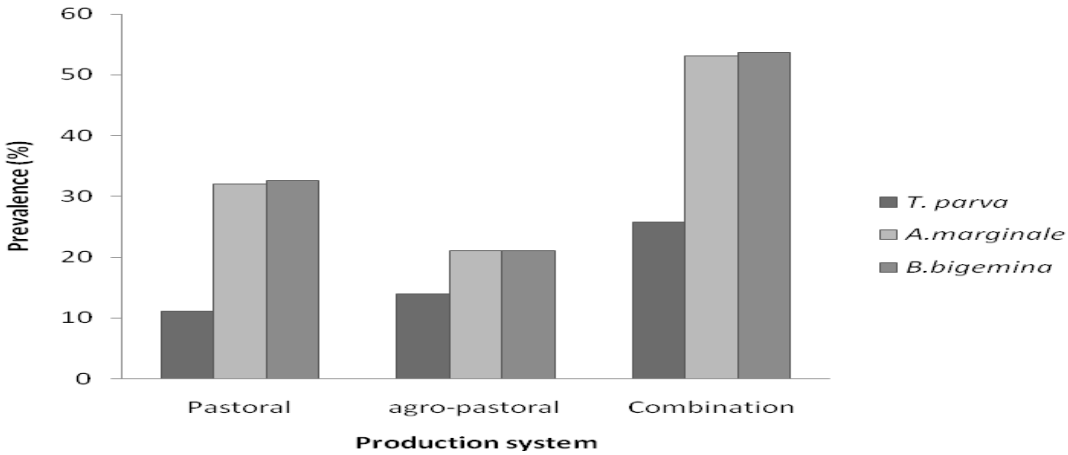


Figure 1: Prevalence of antibodies against *T.parva, A.marginale* and *B.bigemina* in pastoral and agro-pastoral production systems in West Pokot District

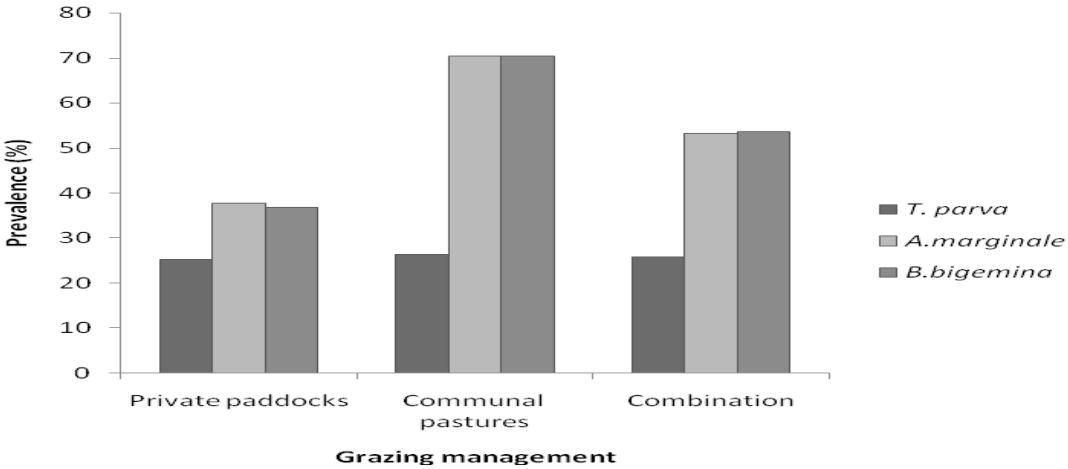


Figure 2: Prevalence of antibodies against *T.parva, A.marginale* and *B bigemina* in different grazing management systems in West Pokot

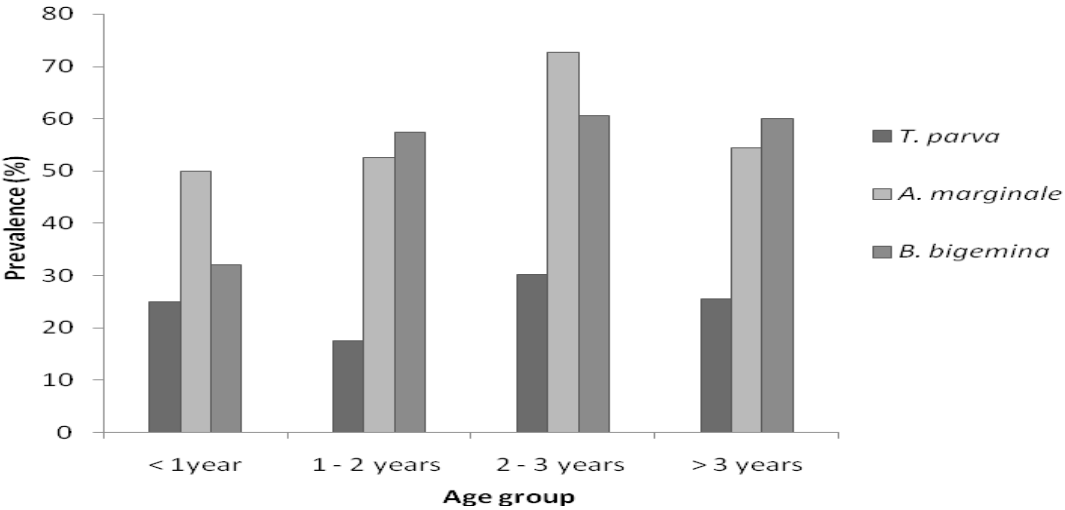


Figure 3: Prevalence of *T. parva, A. marginale* and *B. bigemina* in different age group of cattle in West Pokot District.

including communal boreholes, dams and piped water sources.

Tick control was practiced by all the households with frequencies varying from weekly to only when ticks were judged to be abundant (Table1). The main methods of acaricide application were hand spraying (43.6%), hand washing (30.9%) and communal plunge dips accessed by only 10% of the households. Amitraz (sold in different brand names) was the main acaricide base used by 81.8% of the households. Only 19% of the households were using the acaricide at the manufacturers' recommended mixing regiment. The remaining 82% were using it at either over-strength or under-strength concentrations.

Prevalence of ECF

Serology of 190 samples showed overall prevalence of antibodies to *T. parva A. marginale* and *B. bigemina* was 25.8%, 53.2% and 53.7%, respectively. Overall antibody prevalence for *A. marginale* and *B. bigemina* was higher than those to *T. parva* in both production systems. Similarly, the prevalence of antibodies to *A. marginale* and *B. bigemina* was higher in the different grazing management than for *T. parva* (Figure 1 and 2). The prevalence of antibodies to *A. marginale* and *B. bigemina* was significantly higher in animals in pastoral areas than those in agro-pastoral areas while antibodies to *T. parva* were higher in agro-pastoral than in pastoral areas. Animals grazing on private paddocks had significantly lower prevalence of antibodies against *A. marginale* and *B. bigemina* than those grazing on communal land ($p < 0.05$). There was considerable variation in the prevalence of antibodies to *T. parva, A. marginale* and *B. bigemina* in different age groups (Figure 3). The highest prevalence for all three diseases was in the 2-3 year old animals while the lowest prevalence for antibodies against *A .marginale* and *B. bigemina* was in animals less than one year old. Antibodies against *T. parva* were lowest in 1-2 year old animals. The difference in sero-positivity to *B. bigemina* in the different age groups was significant ($p < 0.05$) whereas the difference in sero-positivity to *T. parva* and *A. marginale* was not significant. Mixed infections (involving presence of antibodies against more than one parasite on the same sample) were detected in a total of 81 samples (42.6%). Approximately half of the mixed infections were double (32.6%) and 8.4% were triple infections. Single infections were detected in a total of 109 samples (57.4%). *T. parva* was detected as single infection in 7 samples (3.7%), *A. marginale,* 27 (14.2%) and *B. bigemina,* 25 (13.2%). A total of 40 (21.1%) samples were negative for all the parasites. Of 176 *Giemsa* stained blood smears examined microscopically, 65 (37.6%) were positive for *Theileria piroplasms* while 10 (5.8%) and 1(0.6%) were positive for *A. marginale* and *B. bigemina* parasites, respectively. None of the lymph node biopsy smears had any *Theileria schizonts*. Mixed infections were detected in two smears, with one smear having *Theileria piroplasms* and *A. marginale* parasites while the other had Theileria and *B. bigemina piroplasms*.

Risk factors associated with serum antibody prevalence.

The results of logistic regression analysis for the variables significantly associated with serum antibody prevalence for the different tick-borne diseases are shown in table 2. Sero-prevalence to *T. parva* was associated with the breed, grazing management and the age of the animal. Crossbred animals were significantly more likely to seroconvert to *T. parva,* than indigenous animals ($p < 0.035$). Animals grazed in private paddocks were significantly less likely to seroconvert than animals grazed in communal pastures. Animals aged less than two years were significantly more likely to sero convert to *T. parva* than other age categories ($p < 0.052$). Sero-positivity to *A. marginale* was only significantly associated with the service provider. Animals that were attended by a veterinarian were significantly more likely to

Table 2: Variables associated with *T. parva, A. marginale* and *B. bigemina* sero-prevalence in cattle from the logistic regression model in West Pokot district.

Variable	b	SE (b)	p-value
T. parva			
Breed (1 = crossbred, 0 = indigenous)	2.47	1.17	0.035
Grazing(1private paddocks, 0 = communal pastures)	-2.43	1.16	0.036
Age-group (1= < 2 years, 0 = other age categories)	0.90	0.46	0.052
A. marginale			
Grazing(0 = private paddocks, 1 = communal pastures)	0.63	0.35	0.074
Vet. service provider (1= professional vet., 0 = other service providers)	1.72	0.78	0.026
B. bigemina			
Age-group (1= < 2 years, 0 = other age categories)	-1.09	0.43	0.010
Production system (0 = pastoral, 1 = agro-pastoral)	0.86	0.36	0.020

seroconvert ($p < 0.0236$) than those attend by other service providers. Sero-positivity to *B. bigemina* was associated with the age of the animal and production system. Animals less than 2 years were significantly less likely to seroconvert to *B. bigemina* ($P < 0.010$) than those in other age categories. Also, animals raised under pastoral production system were significantly more likely to seroconvert than those raised under agro-pastoral production system ($p < 0.020$). No significant interactions were found between risk factors for sero-prevalence for the three tick-borne infections.

Tick species distribution and apparent densities.

Ninety eight percent of the herds in the study were tick infested. Total body count was done on all the animals selected and 1244 ticks were collected for genera and species identification. The following species of ticks were found in the study sites namely *R.appendiculatus, B. decoloratus,* R. evertsi, *Amblyomma spp, Hyalomma spp*, and *R.pulchelus* in order of abundance. Field infestation levels varied across farms and locations within the district. The highest tick infestation was recorded in locations in pastoral areas while lower infestations were recorded in locations in agro-pastoral areas where grazing is mainly on private paddocks and tick control is practiced. The distribution of ticks according to species in the study locations is presented in Figure 4 and 5. The range and number of species found at different sites were markedly different.

Discussion

The objective of this study was to assess the extent to which tick-borne diseases are a constraint to the livelihoods of livestock keepers in the marginal areas of the vector distribution in Northern Rift valley. It was also hoped that the data collected might be used in the development of control strategies if the diseases turned out to be important constraints. In the study area nearly all people depend on agriculture (96%) and mostly on livestock keeping. Areas on higher altitudes had crop cultivation in addition to livestock keeping. Tick-borne diseases were viewed as important as nearly all livestock keepers practiced tick control and since these services are fully paid for by farmers, they must consider the diseases important enough to be willing to pay of the service. The frequency used did not appear targeted at disease control but rather at reducing tick burdens as only 18% of the farmers treated animals weekly. It is not clear if the frequency of tick control was

Table 3: Percentage tick counts and distribution pattern by species in study areas in West Pokot District

	Rhipicephalus spp	Amblyoma spp	Boophilus spp	Hyalomma spp
Kopulio	4.26	2.61	18.94	0.07
Suam	6.14	1.98	1.01	0.22
Lokichar	6.05	1.08	2.1	0.23
Kodich	14.86	1.76	0.15	0.39
Serewo	3.46	1.65	0	0.04
Riwo	3.46	0.39	0.13	0.02
Ywalateke	6.2	0.76	0.63	0.01
Chepkopeigh	4.13	0.2	0.09	0.28
Senetwo	0.8	0.2	0.37	0.02
Kipkomo	2.63	0.04	1.06	0
Batei	3.6	0.59	0	0.17

deliberate or farmers lacked information on appropriate tick control regimens.

The prevalence of antibodies to *A. marginale*, *B. bigemina* and *T. parva* at 53.2%, 53.7% and 25.8%, respectively, were relatively low. However, these levels are lower than what would be expected if the diseases were in a state of endemic stability. Endemic stability describes a state in cattle population where the large majority of the population becomes infected and immune by about six months of age and little or no clinical disease occurs (Norval *et al.,* 1992). The serology results imply clinical diseases from the tick-borne disease are common and might be the reason why tick control is widely practiced. Unfortunately, this level of tick control is unlikely to control the diseases especially ECF. The most likely explanation for the endemic instability in this area is low vector abundance. The area forms part of the northern limit of the distribution of the vectors of the three TBDs (Walker, 1974). Perry and Young (1995) have noted that regions in the margins of the distribution of *R. appendiculatus* are characterized by periodic unsuitability of climate for the tick and therefore, its abundance from year to year. Under such conditions some animals reach adulthood without becoming infected and are therefore, likely to get clinical disease.

Antibody prevalence to *A. marginale* and *B. bigemina* were significantly higher in cattle under pastoral than the agro-pastoral production systems. This is probably the outcome of two opposing processes. Pastoral areas were generally drier than agro-pastoral areas and consequently less suitable for ticks. This would be expected to lead to lower sero-prevalence. On the other hand, there was far less tick control in pastoral areas partly because of lack of service providers but also due to the fact that pastoralists kept only Zebu cattle which they thought exhibited a certain amount of resistance to ticks thus negating the need for tick control. This would tend towards higher sero-prevalence. The final level of sero-prevalence would therefore, depend on the process exerting the greater influence. However, this argument does not appear true for *T. parva* as the prevalence of antibodies was higher in agro-pastoral production system than pastoral systems. This could be an indication that tick control was not as effective against *T. parva* as would have been expected. Nearly 50% of farmers reported that they spray cattle every two weeks. This frequency is not enough to prevent *T. parva* infection since the vector is a three-host tick whereas it is

probably adequate control *A. marginale* and *B. bigemina* infection which are spread by a one-host tick vector. To prevent *T. parva* infection, weekly or even higher frequency of tick control is normally recommended (Norval *et al.,* 1992).

This is an important observation in that where multiple TBDs occur, tick control aimed at controlling diseases should be carefully thought out since one approach may not be adequate for all the diseases. It could well be that the inefficient tick control widely carried out is not adequate to control *T. parva* but is sufficient to control the other TBDs. As expected, antibody prevalence against the three TBDs was lower in animals kept in private paddocks than those on communal pastures. This is most likely because farmers who animals were grazing on private paddocks would also practice more tick control and animals once treated are less likely to be re-infested by other animals. Antibody prevalence generally rose with age of the animals, as expected for antibodies that remain for long periods because of the cumulative effect, except for *T. parva*. Antibodies against *T. parva* for animals aged between 1-2 years were significantly lower than for other age groups. The fact that at one year only 53.2%, 53.7% and 25.8% of the animals are sero-positive for *A. marginale, B. bigemina* and *T. parva*, respectively, is further evidence of endemic instability in the area. Furthermore, the higher level of antibody prevalence to *A. marginale* than *B. bigemina* is further evidence that vector abundance is probably the limiting factor. The higher prevalence of antibodies against *A. marginale* could be because it can be transmitted by other means in addition to tick vectors. Serological findings were corroborated by examination of blood smears. Although it might appear as if the two results are contradictory, the reason is that the tests were measuring different outcomes. *Theileria piroplasms* from some strains of *T. parva* remain for long periods in most animals referred as carriers. *A. marginale* and *B. bigemina* parasites on the other hand are only found in clinical and subclinical cases. Furthermore, although *Theileria piroplasms* were found in 65% of the animals while only 26% of the animals were serologically positive, the blood smears could not have all been *T. parva* because it is very difficult to distinguish microscopically between *T. parva, T. mutans* and *T. taurotragi* the most common theileria in Kenya whereas the ELISA test is specific for *T. parva.*

Whereas a number of epidemiological studies on ECF and other tick-borne diseases have been carried out in the dairy and mixed farming high potential areas of the country, very few, studies are from pastoral and agro-pastoral production systems in marginal areas. Some of these reports have suggested that the climatic conditions in marginal areas are unfavorable for the tick vector responsible for transmission of the disease to thrive and therefore, tick-borne diseases are not important (Perry and Young, 1995). The results of the present study are contrary to that view. Tick-borne diseases are important in these production systems and there is no evidence of endemic stability. It is generally agreed tick control does not provide a long-term solution for TBDs especially in pastoral systems. Enhancing endemic stability through immunization against ECF might seem the better option. This study provides a basis for detailed epidemiologic studies to test such strategies and provide further evidence on the incidence and prevalence of tick-borne diseases, alongside broader quantification of other constraints faced by livestock keepers. In addition, the findings are useful to extension workers in addressing the needs of the livestock keepers.

Impact

The present study improves our understanding of the dynamics of tick-borne infections in marginal areas. It provides further evidence that the diseases are

prevalent and of increasing in importance to pastoralists who rely on livestock production as the only livelihood activity. This is expected to influence policy decisions for arid and semi-arid areas regarding the distribution of the vector ticks and prevalence of cattle diseases which is crucial in assessing the extent of the diseases and their potential impact on cattle and therefore, in planning of control strategies and delivering of technologies to the marginalized pastoral communities.

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References

ALRMP, (2005): West Pokot District Vision and Strategy: 2005-2015 July 2005.

Di Giulio G, Lynen G, Medley G, O'Callaghan C J, Kiara H K, McDermott J J, 2003: Characterization of theileriosis in pastoral production systems in Northern Tanzania. In: Proceedings of the 10th I*nternational symposium for Veterinary Epidemiology and Economics*, Vina del Mar, Chile, 17–21 November 2003.

Dolan T T and Young A S, 1981: An approach to the economic assessment of ECF in Kenya, in: Irvin A.D., Cunningham M.P., Young A.S. (Eds.), Advances in the control of *Theileriosis*, Proceedings of an international conference, 9–13th February, 1981 ILRAD, Nairobi, Martinus, Nijhoff, The Hague, 1981, pp. 412-415.

Government of Kenya, (2003): Economic Recovery Strategy for Wealth and Employment Creation, 2003-07. *Ministry of Finance and Planning*. Government Printing Office, Nairobi.

Horak I G, 1982: *Onderspoort Journal of Veterinary Research.* 1982; 49: 85-93.

Jaetzold R and Schmidt H, 1983: Farm Management Handbook of Kenya, Volume II. Part B, Central Kenya. *Natural conditions and farm management information*, Ministry of Agriculture, Kenya.

Maloo S H, Thorpe W, Kioo G, Ngumi P, Rowlands G J and Perry B D, 2001: *Preventive Veterinary Medicine*. 52, 1–16.

Maloo S H, Thorpe W, Kioo G, Ngumi P, Rowlands G J and Perry B D, 2001: *Preventive Veterinary Medicine*. 52, 1–16.

Norval R A I, Perry B D and Young A S, 1992: *The epidemiology of theileriosis in Africa*. London: Academic Press, 1992: 481 pp.

Ngotho R, McLeod A, Wamwayi H and Curry J, 1999: Economic effects of Rinderpest in pastoralist communities in West Pokot and Turkana. Paper presented to the KARI/DFID NARP II End of Project *Conference, KARI Headquarters,* Nairobi, Kenya, March, 1999.

Otim C P, 2000: *Journal of Agricultural Science*, 5: 79 - 85.

Perry B D and Young A S, 1995): *Preventive Veterinary Medicine*. 25: 107-120.

Rubaire-Akiiki C, Okello-Onen J, Nasinyama G W, Vaarst M, Kabagambe E K, Mwayi M, Musunga D and Wandukwa W, 2004; *Journal of Insect Science*, 4:8,8pp. Available online: insectscience.org/4.8.

Walker J B, Keirans J E, and Horak I, 2000: The *Genus Rhipicephalus* (*Acari, Ixodidae*). Cambridge University Press, UK.

Wright P F, Nelson F, Van Rooij E M A, Lelenta M, Jeggo M H, 1993: Standardization and validation of enzyme-linked immunisorbent assay techniques for the detection of antibody in infectious disease diagnosis. Revue Scientific Technique *Office Internationale Epizootique*, 12: 435–450.

PATHOGENIC EFFECTS ASSOCIATED WITH NATURAL GASTROINTESTINAL HELMINTH INFECTIONS IN PIGS IN KENYA

EFFETS PATHOGÈNES ASSOCIÉS AUX INFECTIONS GASTRO-INTESTINALES NATURELLES DUES À L'HELMINTHE CHEZ DES PORCS AU KENYA

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Abstract

Pathogenic effects associated with gastrointestinal helminths infections in pigs were analyzed in 78 infected and 37 non-infected pigs in Department of Veterinary Pathology and Microbiology, University of Nairobi. Gross and microscopic lesions encountered were scored subjectively and the results compared using Mann-Whitney rank sum test. Of the infected, 37 (47.4 %) had mild lesions in gastrointestinal tract while the remaining 41 (52.6%) and all non-infected pigs had no lesions. Catarrhal gastritis was observed in *Ascarops strongylina* (2), while in mixed *Physocephalus. Sexulatus* and *Hyostrongylus rubidus*, focal mucosal necrosis was an additional lesion. Partial intestinal obstruction (2), muco-purulent enteritis (3), villous atrophy (2) and entrapped larval cysts (1) were encountered in small intestines of pigs infected by Ascaris suum, *Trichostrongylus columbriformis*, mixed *A. suum, T. columbriformis* and *Strongyloides ransomi*; *T. columbriformis* and *S. ransomi* and *A. ransomi,* respectively. In large intestines, suppurative typhlocolitis (13) and nodular lesions were observed in *Trichuris suis* and *Oesophagostomum spp* infected pigs, respectively. *Allergic hepatitis* (11) and *pneumonia* (2) were extra-intestinal lesions associated with migratory *A. suum* larvae. In the study, gastrointestinal helminths infections were associated with mild *pathogenic* effect, however, their contribution to overall pig performance and organ condemnation at slaughter requires further investigations.

Key words: Natural *helminth* infections, Kenyan pigs.

Résumé

Les effets pathogènes associés aux infections gastro-intestinales dues à l'helminthe chez les porcs ont été analysés chez 78 porcs infectés et 37 porcs non infectés dans le Département de pathologie et de microbiologie vétérinaires de lUniversité de Nairobi. Les lésions macroscopiques et microscopiques constatées ont été notées selon des critères subjectifs et les résultats ont fait l'objet de comparaisons sur la base du test de la somme des rangs de Mann-Whitney. Parmi les porcs infectés, 37 (soit 47,4% de l'ensemble) présentaient des lésions légères dans le tube digestif alors que les 41 autres (52,6%) et le reste des porcs non infectés ne présentaient aucune lésion. La gastrite catarrhale a été observée dans les infections à Ascarops strongylina (2), tandis que dans les infections mixtes à Physocephalus, à Sexulatus et à Hyostrongylus rubidus, l'on a relevé , comme lésion supplémentaire, des cas de nécrose focale de la muqueuse. Les chercheurs ont également eu à constater des cas d'occlusion intestinale partielle (2), dentérite muco-purulente (3), d'atrophie villositaire (2) et de kystes larvaires piégés (1) dans l'intestin grêle des porcs infectés par Ascaris suum, Trichostrongylus columbriformis, ainsi que des cas d'infections mixtes à A. suum, T. columbriformis et Strongyloides ransomi ; à T. columbriformis et S. ransomi et à A. ransomi, respectivement. Dans le gros intestin, les

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analyses ont permis de constater des cas de typhlocolite suppurative (13) et des lésions nodulaires chez les porcs infectés par Trichuris suis et par Oesophagostomum spp, respectivement. Les cas d'hépatite allergique (11) et de pneumonie (2) étaient des lésions extra-intestinales associées à des larves A. suum migratrices. Dans le cas de la présente étude, les infections gastro-intestinales dues à l'helminthe ont été associées à l'effet pathogène doux ; cependant, leur contribution à la performance globale du porc et la condamnation des organes du porc lors de l'abattage nécessitent des travaux de recherche complémentaires.

Mots clés: Helminthiases naturelles ; porcs du Kenya.

Introduction

Gastrointestinal helminthoses in pigs occasionally cause obvious disease and death, but more commonly, they lead to loss of appetite, reduced weight gain, poor feed utilization, potentation of other pathogens and condemnation of parts of the carcasses at slaughter (Wakelin, 1984). These effects of parasitism originate from pathologic alterations of tissue structure and function some of which are well documented as a result of controlled single-species experimental infection (Soulsby, 1982). However, under natural conditions, most pigs have mixed infections (Walkelin, 1984, Ng'ang'a et al., 2008), but their outcomes are largely unknown.

In Kenya, potentially pathogenic *Oesophagostomum species*, *Trichuris suis*, Ascaris suum, *Trichostrongylus* species, *Strongyloides ransomi*, *Hyostrongylus rubidus*, *Ascarops strongylina* and *Physocephalus sexulatus* are prevalent in pigs (Kagira *et al.,* 2002,Wabacha *et al.* 2004, Ng'ang'a *et al.,* 2008). Developmental stages of some of these parasites penetrate the mucous membrane thereby compromising the defense mechanisms and often leading to secondary bacterial infections (Mansfield and Urban, 1996, Mansfield *et al.,* 2003). The ensuing inflammatory reaction may interfere with the process of digestion or utilization of products of digestion. Other larvae may penetrate through the intestinal wall to cause *peritonitis*, hepatic lesions, pneumonia among others; leading to ill-health or condemnation of affected organs at slaughter (Matusyavichus, 2003). On the other-hand, adult worms not only compete for feed with the host, but some like *A. suum* may become entwined within the lumen to cause intestinal obstruction.

Inspite of all these possibilities, the *pathogenic* effects associated with natural infections in Kenyan pigs are largely unknown, yet it is acknowledged that they cause significant economic losses (Kagira *et al.,* 2002, Wabacha *et al.,* 2004). This study therefore aimed at analyzing the gastrointestinal and extra-intestinal *lesions* associated with natural helminths infections in pig carcasses presented for post mortem examination and gastrointestinal tracts collected from slaughter houses.

Materials and methods

A hundred and fifteen pigs used in a previous prevalence study (Nganga *et al.* 2008) comprising 78 infected with gastrointestinal helminths and 37 non-infected are reported in this study. The pigs were subjected to thorough systematic post mortem examination and morbid changes recorded. Samples for histopathology were taken from various levels of gastrointestinal, respiratory and urinary tracts, lympho-reticular organs, nervous organs, liver, heart and endocrine organs with or without gross lesions. These samples were fixed in 10% formalin, dehydrated in graded alcohol, embedded in paraffin wax, sectioned at 4 µm thickness, stained with hematoxylin and eosin and examined using a light microscope.

The pathogenic effects observed grossly and/or microscopically in each part of the gastrointestinal tracts were evaluated in relation to parasite(s) recovered and identified as described in the prevalence

study (Ng'ang'a *et al.*. 2008). The lesions were scored from zero to 2 depending on their severity. A score of zero was allocated to a case where no gross and microscopic lesions were found while a score of 1 was awarded to pigs with either mild lesions comprising acute inflammation of mucus membrane, focal areas of necrosis, hemorrhage or fibrosis. A score of 2 was given to severe reactions manifested as either diphtheritic inflammation of the mucosa, multifocal to diffuse necrosis, hemorrhages or fibrosis in chronic cases. The lesion scores in infected pigs in a given organ were transformed into their natural logarithms, summated and compared with those of non-infected pigs

Table 1: The distribution of *gastrointestinal helminth* infections in the 78 pigs examined and the distribution of lesions associated with them

Organ/organ combinations	Number of pigs infected	Number of pigs with lesions
Stomach only	2	1
Stomach and small intestines	1	0
Stomach and large intestine	3	1
Stomach, small and large intestines	3	1
Small intestines only	9	1
Small intestines and large intestines	19	6
Large intestines only	41	27
Total	78	37

Table 2: The Gross and microscopic lesions observed in the stomach, small intestines and large intestines of pigs naturally infected with *gastrointestinal helminth* parasites

Lesion score	Organ	Helminth parasite involved	Number affected	Gross and/or microscopic lesions observed
1	Stomach (n=3)	*A. strongylina*	2	Hyperemia and increased mucus production
		H rubidus + *P sexulatus*	1	Linear tracts of necrosis and mononuclear infiltration
1	Small intestines (n=8)	*A. suum*	2	Partial intestinal obstruction
		A suum + *T columbriformis* + *S ransomi*	1	Villous atrophy and mucosal neutrophilic infiltration
		T columbriformis + *S ransomi*	1	Villous atrophy and mucosal neutrophilic infiltration
		T columbriformis	3	Focal mucosal necrosis and neutrophilic infiltration
		S ransomi	1	Larval cysts in lamina propria and hyperplasia of crypts
	Large intestines (n=34)	*T. suis*	12	Circulatory disturbances (hemorrhages and congestion)
		T. suis + *Oesophagostomum spp*	6	Nodular intestinal wall with eosinophilic infiltration (5); congestion (1)
		Oesophagostomum spp	16	Nodular intestinal wall with eosinophilic infiltration in lamina propria and submucosa

using Mann Whitney-Wilcoxin rank sum test (GenStat® 2005).

Results

Gastrointestinal helminths infections in the 78 infected pigs were distributed in organ and/or organ combinations as shown in Table 1 and the types of lesions associated with these parasites in any given organ(s) are also shown in Table 2.

In the stomach, mild lesions (score 1) comprising *hyperemia* and *catarrhal* inflammation of gastric mucosa were observed in *Ascarops stronglina* (2) and mixed *Hystrongylus rubidus* and *Physocephalus sexulatus* (1) infections. In addition, linear tracts of necrosis and presence of mononuclear cells mainly lymphocytes were observed in the mucosa in the latter case. Other six pigs infected by *Trichostrongylus axei* (5) and *A. stronglina* (1) had no lesions in their stomachs.

Partial obstruction of the small intestines whereby 35 and 40 adult worms were entwined into ropes was encountered in two pigs infected by *Ascaris* suum. A mild reaction comprising hyperemia and increased mucus production was also encountered in 3 pigs infected by *Trichostrongylus columbriformis*. Microscopically, focal areas of necrosis accompanied by infiltration of neutrophils just below the epithelium were also seen. Subtotal *villous atrophy* of the *duodenum* and proximal parts of jejunum were observed in mixed infections of *A suum*, *T. columbriformis* and *S. ransomi* (1) and *T. columbriformis* and *S. ransomi* (1). Larval cysts accompanied by heavy congestion, necrosis and masses of neutrophils in the lamina propria were obtained in a pig infected by *S. ransomi* in addition to hyperplasia of crypt epithelium. Twenty three other pigs harboring *A. suum* (14), *T. columbriformis* (5) and mixed *A. suum* and *T. columbriformis* (4) parasites had no lesions in the small intestines that could be associated with them.

In the large intestines, mild lesions comprising circulatory disturbances namely petechial hemorrhages and/or mucosal congestion were encountered in 12 pigs infected by *Trichuris suis*. In these pigs, the intestinal contents were loose and pasty. Another pig with mixed *T. suis* and *Oesoghagostomum spp* infection showed congestion as the main gross lesion. Microscopically, varying degrees of suppurative typhlocolitis whereby neutrophils infiltrated the lamina propria just beneath the epithelium was observed in these 13 pigs. In addition, sections of helminth parasites embedded in the mucosa were encountered in a mixed *T. suis* and *Oesoghagostomum* spp infected pig (1), but no inflammatory reaction was observed in the immediate vicinity.

Sixteen pigs infected by *Oesoghagostomum spp* and 5 by a mixture of *Oesoghagostomum spp* and *T. suis* had grossly visible nodules on the mucosa of the caecum and colon. The nodules measured a few millimeters to 10 mm in diameter and their centres looked like "volcano craters" whose floors were covered by creamy material. Microscopically, these nodules comprised lymphoid tissues which had expanded with crater-like pores opening to intestinal lumen, but devoid of an epithelial covering. The cellular population in these nodules comprised blast cells, lymphocytes, macrophages and occasional eosinophils. Presence of eosinophils in the lamina propria and submucosa were constant findings in *Oesophagostomum spp* infected pigs. Another 28 pigs infected by *T. suis* (12), *Oesoghagostomum spp* (9) or a combination of both did not show any gross or microscopic lesion. Lesion scores in stomach, small intestines and large intestines when compared with those of non-infected pigs using Mann Whitney-Wilcoxin rank sum test had p values of 0.92, 0.46 and 0.27, respectively. The p values were >0.25, hence, not significant.

Extra-intestinal lesions were encountered in the liver (11) and the lungs (2). White (milk) spots were distributed all-over the liver surface and permeated

to parenchyma below but in one, the liver capsule was thick and opaque. The lung lesion comprised red and grey portions of consolidation involving mainly the diaphragmatic lobes. Microscopically, the tracts of *fibrous* connective tissue into which many eosinophils had infiltrated were observed both in the liver and lungs. In addition, eosinophils were also found in *interalveolar septae*, walls of bronchioles, peribronchiolar lymphoid tissues and exudates in alveoli and terminal bronchioles.

Discussion

The study revealed that 37 of the 78 pigs infected (47.4%) with gastrointestinal helminths had mild lesions consistent with such infections while the remaining 41 infected (52.6%) and all the 37 had no lesions. The lack of lesions as well as the mild lesions in majority of the pigs may be partly attributed to low - moderate worm counts (Wakelin, 1984). Alternatively, the affected pigs may modulate such infections possibly through inherent genetic factors as studies elsewhere have also shown that some pigs display a strong genetic resistance to *A. suum* and *T. suis* (Nejsum *et al*., 2009). Gastric lesions in pigs infected by gastrointestinal parasites ranged from none in *T. axei* infections to mild non-suppurative gastritis in *A. stronglina* and in mixed H. rubidus and *P. sexulatus* infections. Through burrowing into the gastric mucosa, an acute inflammation, severe in calves and occasionally in pigs associated with *T. axei* was missing in the study (Soulsby, 1982). However, the non-suppurative gastritis observed in *A. stronglina*, *H. rubidus* and *P. sexulatus* infections if extensive may be associated with high serum pepsinogen levels which subsequently interfere with digestion and productivity (Banga-Mboko *et al.,* 2003).

In the small intestines, only 8 out of the 32 infected pigs (25%) had mild lesions and the remaining 24 infected pigs (75%) had no lesions. Though these results may imply that the parasites encountered are well tolerated by the affected pigs, *A. suum* has been associated with intestinal obstruction more severe than observed in this study (de Silva et al., 1997, Stewart and Hoyt, 2006). *Trichostrongylus columbriformis* as observed in the study is accompanied by suppurative enteritis commonly associated with larvae migrating in the *mucosa* (Soulsby, 1982) while a combination of T. *columbriformis*, *S. ransomi* and /or *A. suum* cause villous atrophy, entrapped larval cysts, crypt epithelial hyperplasia and/or lymphoid hyperplasia. All the three parasites are capable of causing these *lesions* individually, but none of the single-species infections had such, suggesting that these lesions may have arisen from additive effects of the combination(s) (Walkelin, 1984, Stewart and Hoyt, 2006).

Many (31) pigs in the study were infected by *A. suum,* but *allergic enteritis* was not a feature because as observed elsewhere, the parasite is able to modulate the host's immune responses (McConchie *et al.,* 2006, Oshiro *et al.,* 2006). However, in extra-intestinal areas, migratory *larvae* cause hemorrhagic foci in the liver and lungs. In other pigs, when these organs are repeatedly exposed to the larvae, fibrous connective tissues infiltrated by eosinophils appear in the liver and lungs (Stewart and Hoyt, 2006) similar to those observed in this study. These lesions do not only cause death in pigs, but also severe scarring, ill-health and condemnation of affected organs at slaughter (Wagner and Polley, 1997).

Trichuris suis and a mixed *T. suis* and *Oesoghagostomum spp* infected pigs in the study were accompanied by circulatory changes and varying degrees of suppurative typhlocolitis. Such lesions are associated with destruction of intestinal epithelium, ulcer formation, bleeding from exposed capillaries and secondary *bacterial* infection (Jubb *et al.,* 1985, Stewart and Hoyt, 2006). *Oesoghagostomum* spp infections in the present study had nodular lesions in caecum and colon that comprised lymphoid

tissues hyperplasia which opened directly into intestinal lumen and an indication of enhanced immune response. This is unlike sheep (Soulsby, 1982) and man (Storey *et al.,* 2000, Boggers *et al.,* 2001) where such nodules represent entrapped *larvae* which form abscesses or calcified cysts. These lesions to some extent interfere with absorption of nutrients and hence reduce productivity in affected pigs.

Gastrointestinal helminth parasites affecting pigs in Kenya as shown in this study might cause mild pathogenic effects. However, poor nutrition (Wabacha *et al.,* 2004), multiple parasites and/or pathogens (Nganga *et al.* 2008) may operate in an animal at the same time leading to additive effects which might cause reduced productivity as speculated in earlier reports (Wabacha *et al*. 2004). It is therefore recommended that further work be carried out on farms where such multiple factors exist to apportion effects of each factor.

Impact

One hundred and fifteen pigs, 78 infected and 37 none infected by worms in digestive canal, were examined with naked eye and using a microscope in order to see damage caused by worms which may interfere with pig performance. Thirty seven (47.4 %) of infected pigs had minor injuries in either stomach and/or intestines that could interfere with digestion in the stomach, movement of food in small intestines and absorption of digested food and water in small and or large intestines. The effects of these injuries on pig performance could not be ascertained in the study and needs further investigations.

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References

Banga-Mboko H, Tamboura H, Maes D, Traore H, Youssao I, Sanglid P T, El Amiri B, Bayala B, Remy B and Beckere J F, 2003. Survey of *gastric lesions* and blood *pepsinogen* levels in pigs in Burkina Faso. *Veterinary Research Communications,* 27: 595-602.

Bogers J J, Storey P A, Faile G, Hewitt E, Yelifari L, Polderman A and Van Marck E A, 2001. Human *oesophagostomiasis*: a *histomorphometric* study of 13 new pigs in northern Ghana. *Virchows Archive,* 439: 21-26.

de Silva N R, Guyatt H L and Bundy D A, 1997. Morbidity and mortality of Ascaris-induced intestinal obstruction. *Transcations of Royal Society of Tropical Medicine and Hygiene*, 91: 31-36.

Jubb K V F, Kennedy P C and Palmer M, 1985. *Pathology* of domestic animals. *New York, Academic Press Inc.*

Kagira J M, Kanyari P W N, Munyua W K and Waruiru R M, 2002. The prevalence of *gastrointestinal* parasites in commercial pig farms in Thika district, Kenya. *Bulletin of Animal Health and Production in Africa,* 50: 1-11.

Mansfield L S and Urban J F Jr, 1996. The *pathogenesis* of *necrotic* proliferative colitis in swine is linked to whipworm induced suppression of *mucosal* immunity to resident bacteria. *Veterinary Immunology and Immunopathology,* 50: 1-17.

Mansfield L S, Gauthier D T, Abner S R, Jones K M, Wilder S R and Urban J F, 2003. Enhancement of disease and pathology by synergy of *Trichuris suis* and *Campylobacter* jejuni in the colon of immunologically naïve swine. *American Journal of Tropical Medicine and Hygiene,* 68: 70-80.

Matusyavichus A P, 2003. Associated disease caused in pigs by Ascaris and *Oesophagostomum*. *Parazitotsenozy I assotsiativyne bolezni,* 16: 219-234

McConchie B W, Norris H H, Bundoc V G, Trivedi S, Boesen A, Urban J F Jr and Keane-Meyers A M, 2006. *Ascaris suum-derived* product suppress *mucosal allergic* inflammation in an interleukin-10-independent manner via interference with dendritic cell function. *Infection and Immunity*, 74: 6632-6641.

Nejsum P, Roepstorff A, Jorgensen C B, Fredholm M, Goring H H, Anderson T J C and Thamsborg S M, 2009. High heritability for *Ascaris* and *Trichuris* infection levels in pigs. Heredity, 102: 357-364

Ng'ang'a C J, Karanja D N and Mutune M N, 2008. The prevalence of *gastrointestinal* helminth infections in pigs in Kenya. Tropical *Animal Health and Production,* 40: 331-334.

Oshiro T M, Enobe C S, Araujo C A, Macedo M S and Macedo-Soares M F, 2006. PAS-1, a protein affinity purified from *Ascaris suum*, maintains the ability to modulate the immune response to a bystander antigen. Immunology and Cell Biology, 84: 138-144.

Soulsby E J L, 1982. Helminths, arthropods and protozoa of domesticated animals, Bailliere Tindal, *English Language Book Society.*

Stewart T B and Hoyt P G, 2006. Internal parasites. In Diseases of Swine, Eds; Straw B E, Zimmermann J J, D'Allaire S and Taylor D J, Ames Iowa, *Blackwell publishing,* pp: 901-914.

Storey P A, Faile G, Hewitt E, Yelifari L, Polderman A M and Magnussen P, 2000. Clinical *epidemiology* and classification of human oesophagostomiasis. *Transactions of Royal Society of Tropical Medicine and Hygiene,* 94: 177-182.

Wabacha J K, Mulei C M, Kyule M N, Zessin K H and Mbithi P M, 2004. *Helminthosis* in smallholder pig herds in Kikuyu division, Kiambu district, Kenya. *Kenya Veterinarian,* 26: 29-33.

Wagner B and Polley L, 1997. Ascaris suum prevalence and intensity: an abattoir survey of northern Saskatchewan, *Veterinary Parasitology,* 73: 309-13.

Wakelin D. 1984. Immunity to parasites. London, Wakelin D and Edward A Limited.

HAEMATOLOGICAL, CARCASS CHARACTERISTICS AND MEAT QUALITY OF INTENSIVELY MANAGED WEST AFRICAN DWARF GOATS SLAUGHTERED AT DIFFERENT LIVE WEIGHTS

CARACTÉRISTIQUES HÉMATOLOGIQUES DES CARCASSES ET QUALITÉ DE LA VIANDE DES CHÈVRES NAINES DE L'AFRIQUE DE L'OUEST ÉLEVÉES SOUS LE RÉGIME D'EXPLOITATION INTENSIVE ET ABATTUES DANS DES CONDITIONS DE POIDS VIF DIFFÉRENTES

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Abstract

The West African Dwarf (WAD) breed of goats was slaughtered at live weights (LW) of 10, 12, 14, and 16kg to determine the effect of live weight at slaughtering on the carcass characteristics and meat quality. Prior to slaughtering, blood samples were collected through jugular vein of each animal to determine the haematological parameters. The haematological parameters monitored varied ($P < 0.05$) across the treatments with goats slaughtered at 10 to 12kg LW having significantly ($P < 0.05$) lower values with exception of blood glucose and total protein. Carcass characteristics also differed significantly ($P < 0.05$) with goats slaughtered at 14 and 16kgLW having significantly ($P < 0.05$) higher values. DM contents of the meat was not influenced ($P > 0.05$) by LW at slaughter but crude protein, fat, ash contents and pH values differed significantly ($P < 0.05$) across the treatments. In sensory evaluation, panelists rated texture and juiciness the same ($P > 0.05$), while colour of meat from goats slaughtered at 10kg LW was rated low ($P < 0.05$). Meat of goats slaughtered at 10 and 12kg LW was more tender ($P < 0.05$) compared with that slaughtered at higher LW while overall acceptability was rated high ($P < 0.05$) for goats slaughtered at higher LW. It was however concluded that the haematological parameters, carcass characteristics and meat quality of West African dwarf goats differ with LW at slaughter. Goats slaughtered at 14 and 16kg LW produced high DP and their meats were most acceptable to the panelist on account of preference.

Key words: Goats, live weight at slaughter, haematological, carcass, meat quality

Résumé

Les chèvres de race naine de l'Afrique de l'Ouest à poids vifs différents compris entre 10, 12, 14 et 16 kg ont été abattues aux fins de détermination de leffet du poids vif à labattage sur les caractéristiques de la carcasse et la qualité de la viande. L'on a procédé, avant labattage, au prélèvement des échantillons sanguins à travers une incision de la veine jugulaire de chaque animal en vue de définir les paramètres hématologiques. La surveillance des paramètres hématologiques a permis de constater des variations ($P <0,05$) à travers l'examen des données d'analyse des chèvres abattues dont les poids vifs étaient compris entre 10 et 12 kg. Leurs caractéristiques naturelles sensiblement très faibles ($P <0,05$) ont été notées, à lexception de la glycémie et de protéines totales. Les caractéristiques des carcasses présentaient également des différences tout à fait évidentes ($P <0,05$) notamment chez les chèvres abattues dont les poids vifs étaient compris entre 14 et 16 kg et présentant des caractéristiques naturelles ($P <0,05$) très élevées. La teneur de la viande en DM n'a pas été affectée ($P> 0,05$) par le poids vif à labattage, mais les teneurs en protéines brutes, en matières grasses, en cendres et les valeurs de pH faisaient ressortir, par contre, des différences appréciables ($P <0,05$) entre les traitements. Dans lévaluation sensorielle, les chercheurs ont noté

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que la composition et la jutosité de la viande comportaient des valeurs similaires (P> 0,05), tandis que la couleur de la viande provenant des chèvres abattues à poids vif de 10 kg a été jugée fade (P <0,05). La viande des caprins abattus à poids vifs compris entre 10 et 12 kg était plus tendre (P <0,05) par rapport à ceux abattus à poids vifs plus élevés, alors que l'acceptabilité globale a été jugée élevée (P <0,05) pour des chèvres abattues à poids vifs plus élevés. Dans leurs conclusions, les chercheurs ont, toutefois, affirmé que les paramètres hématologiques, les caractéristiques des carcasses et la qualité de la viande des chèvres naines de lAfrique de lOuest différaient en fonction de leurs poids vifs à labattage. L'équipe de chercheurs a noté, chez les chèvres abattues dont les poids vifs variaient entre 14 et 16 kg, un DP élevé et une qualité de viande beaucoup plus appréciable selon leurs critères de préférence.

Mots clés: Chèvres, poids vif à labattage, hématologiques, carcasse, qualité de la viande.

Introduction

Goats are important animals in subsistence agriculture in Nigeria. The West African dwarf (WAD) goats are predominantly found in the southern part of Nigeria, which is humid and favours high prevalence of diseases (Oni, 2002). The eco-zone is infested with tsetse fly and the dwarf goat thrive well and reproduce with twins and triplet births (responsible for their high economic turnout rate) in the ecological niche (Adeloye, 1998), thereby satisfying a part of the meat requirement in this region. Goat meat has been established as lean meat with favourable nutritional quality. Its attributes are concordant with present day consumer demands for leaner and nutritious meat (Webb *et al.*, 2005)

Goats in Nigeria are usually slaughtered at various weights at the owners' discretion which results differing carcass yield and quality. The preference for goat meat may be due to special features, which makes it more organoleptically different from mutton, and on weight basis goat meat has higher lean meat content than mutton and less wastage (Devendra and Owen, 1983). The relative importance of goat meat in the tropics is associated with its wide distribution especially on small farm holdings where goats play an important role in contributing meat and milk as well as providing some income (Adu *et al*. 1996). Since WAD goat production in south western Nigeria is focused on the breed and because of its numerical importance, there is need to quantify their health status and carcass characteristics. The present study was conducted to provide primary information on the haematological parameters which is a fair indication of whether animals are oversupplied or undersupplied with nutrients, as well as for diagnosing the production status of these animals (Daramola *et al*., 2005), and also the carcass characteristics as well as the meat quality of WAD goats managed intensively and to examine the changes in composition that may be influenced by live weight at slaughter.

Materials and methods

Sixteen (16) West African Dwarf male goats of different live weights ranging from 10 to 16kg managed under the intensive system at the Teaching and Research farms of the University of Agriculture, Abeokuta, Ogun State, Nigeria were used for the study. The goats were randomly allocated into four treatments of four replicates, balanced for weight in a completely randomized design. They were fed fresh *Panicum maximum ad-libitum*, supplemented with 17% concentrate with water provided *ad-libitum*. A day to slaughtering, blood samples were collected from the jugular vein into lithium herparin tubes and centrifuged at 3000rpm for 15 minutes at 4^0C. Plasma was harvested and stored at -20^0C until analyzed.

The goats were then fasted for 24hours, weighed and slaughtered at various predetermined weights of 10, 12, 14 and

16kg according to the local method of severing the jugular veins, throat and trachea without stunning. After thorough bleeding, the hair was scalded from the skin using boiling water. Empty body weight was computed by subtracting the weight of the gut content from the slaughter weight. The hot carcass weight was weighed by removing the head, feet and gastric intestinal tract within one hour of slaughter with organs being carefully excised, weighed and chilled for 24 hours at 4°C, and then weighed again to determine the cold carcass weight. The carcasses were cut into retail parts (shoulder, rack, loin, legs, neck/breast, and shank/flank) and each part was weighed as described by Adu and Brinckman (1981) and calculated as percentage of empty body weight. The dressing was calculated as the ratio of cold carcass weight to live weight in percentage.

In evaluating the sensory qualities, samples of meat from loin in each treatment were collected, tagged for identification, put in a double layered polythene bags and cooked in water at 80°C for 40 minutes in a pot. Ten panelists were trained in the assessment procedure and were subsequently required to masticate on a sample each from each treatment and score it for flavour, tenderness, juiciness and overall degree of acceptability (Iwe, 2002). The evaluators scored each sample on a nine (9) points category rating scale (9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like or dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely) (AMSA, 1978) for colour, juiciness, flavour, texture, tenderness while overall acceptability was scored on a 3 point scale (1 = least acceptable; 2 = more acceptable and 3 = most acceptable).

Chemical analysis

The Dry matter content of the meat was determined by oven drying at 1000C for 24hours, crude protein by Kjeldahl method, fat by Soxhlet extraction using petroleum ether, and ash content was measured through ashing in a furnace at 500^{0}C for 6 hours (AOAC, 1990). Meat sample (10g) homogenized in distilled water (90ml) was used to measure the pH value using pH meter (Ockerman, 1985).

Statistical analysis

Data generated were based on completely randomized design and subjected to one way analysis of variance using the statistical package (SAS, 1999). Significant means were separated using Duncan Multiple Range Test (Duncan, 1955).

Results and Discussion

The chemical composition of feed fed the experimental goats is shown in Table 1. The chemical composition of *Panicum maximum* is comparable to that reported by (Bamikole *et al.,* 2001) and higher than that reported by (Fasae *et al.*, 2009). Feeding *Panicum maximum* supplemented with concentrate has been found to support growth in goats (Isah *et al.*, 2007).

The haematological parameters of WAD goats differed significantly ($P < 0.05$) across

Table 1: Composition of diets (%) fed West African dwarf goats slaughtered at different weights

Ingredients	Concen-trate	Panicum maximum
Wheat offal	40	-
Corn offal	35	-
Palm kernel cake	22	-
Bone meal	2	-
Mineral /salt mixture	1	-
Total	100	
Determined analysis (%)		
Dry matter	85.54	89.82
Crude protein	17.20	9.14
Crude fibre	12.11	3.65
Ether extract	3.63	1.64
Ash	9.35	13.86

Table 2: Haematological parameters of West African Dwarf goats slaughtered at different live weights

Parameters	Slaughter Weights (Kg)				
	10	12	14	16	SEM
Packed cell volume (%)	24.55[b]	25.60[b]	28.50[a]	31.40[a]	0.72
Red blood cells (µ/L)	12.10[b]	12.30[b]	13.40[a]	13.95[a]	0.34
White blood cells (µ/L)	11.41[b]	11.94[b]	12.65[a]	13.32[a]	0.22
Blood urea nitrogen (mmol/L)	3.71[b]	3.95[b]	4.45[ab]	4.94[a]	0.16
Glucose (mmol/L)	3.11	3.07	3.45	3.57	0.12
Total protein (mmg/L)	5.85	5.90	5.73	5.80	0.21

[a,b,c] Mean values on the same row with same superscripts are not significantly (P >0.05) different

the weight groups (Table 2). The packed cell volume (PCV) observed in this study were higher than values reported for the same breed of goat (Orheruata *et al.,* 2004). This might be attributed to the intensive system of management practiced with goats in this study which could be responsible for proper maintenance of their PCV. Grazing goats have earlier been reported to have a reduction in PCV of about 9 to11% due to grazing stress and in sufficient protein intake (Blood et al., 1989). However, goats slaughtered at higher live weights (LW) had significantly ($P < 0.05$) higher PCV values compared with goats with lower weights. The values obtained for red blood cells and white blood cells (WBC) were higher ($P < 0.05$) in heavier goats. WBC values among the treatments compared well with the range obtained for WAD goats (Daramola *et al*., 2005; Fasae *et al.,* 2005) and higher than the range reported for Red Sokoto goats (Tambuwal *et al.,* 2002). This suggests that WAD goats possess protective mechanism thereby providing a rapid and potent defense against infectious agents, which confirms their ability to thrive well and reproduce in the eco zone infested with tsetse fly (Adeloye, 1998; Oni, 2002). The values obtained for blood glucose level and total protein were similar ($P > 0.05$) across the weight groups and were within the range reported for goats (Puls, 1994) under different management systems.

The carcass characteristics of WAD goats in this experiment varied ($P < 0.05$) across the weight groups (Table 3). Carcass characteristics were found to increase with live weight as heavier goats dressed higher ($P < 0.05$) than the lighter goats by about 2 to 3%. This is in consonance with the findings of Singh et al. (1994) that reported a significant increase in carcass characteristics of Bengal goats due to LW at slaughter. However, differences were not observed in the percentages of the retail components across the weight groups. The dressing percentage (DP) of goats slaughtered at 14 and 16 kg LW were similar but higher ($P < 0.05$) compared with the other weight groups. However, DP has been reported to be influenced by age, weight, sex, body condition, amount of gut fill at slaughter, whether the carcass is weighed hot or cold and, of course, by the number of body components included in the yield calculation (Pike *et al.,* 1973). Marichal *et al.,* (2003) also observed variation in DP of carcass from

Table 3: Carcass characteristics of West African Dwarf goats slaughtered at different live weights

Parameters	Slaughter Weights (Kg)				
	10	12	14	16	SEM
Slaughter weight (kg)	10.5d	13.5c	15.0b	16.5a	1.04
Empty body weight (kg)	9.11d	12.14c	13.6b	14.78a	0.88
Carcass weight (kg)	4.08b	5.38b	6.10a	6.92 a	0.47
Hot carcass weight (kg)	6.42c	7.89b	9.32ab	9.72a	0.15
Dressing percentage (%)	38.86b	39.85ab	40.67a	41.94a	0.52
Retail cuts (% EBW)					
Leg	22.2	21.8	21.6	21.6	0.13
Loin	13.8	14.3	15.0	15.7	0.14
Shoulder*	14.8	15.4	14.8	14.1	0.09
Rack**	13.5	12.4	12.8	13.2	0.11
Neck/breast	21.3	20.7	21.0	21.1	0.07
Shank/flank	14.4	14.7	14.1	13.9	0.08

[a,b,c] Mean values on the same row with same superscripts are not significantly (P >0.05) different
EBW - Empty body weight
*Shoulder - 5 rib shoulder
**Rack – Included the 6 – 12 ribs.

Table 4: Chemical composition (%) of meat* from West African Dwarf goats slaughtered at different live weights

Parameters	Slaughter weights (Kg)				
	10	**12**	**14**	**16**	**SEM**
Dry matter	35.12	35.67	35.80	35.64	1.04
Crude protein	26.91[c]	28.04[b]	28.91[b]	30.32[a]	0.88
Ether extract	7.91[b]	7.54[b]	8.65[a]	8.97[a]	0.47
Ash	6.11[b]	6.25[b]	6.95[ab]	7.14[a]	0.15

[a,b,c] Mean values on the same row with same superscripts are not significantly (P >0.05) different
*Meat from loin

Canary breed of goats based on live weight. The carcass yield and characteristics of WAD goats among the weight groups in this study were however, similar to those reported for brown Bengal goats (Singh *et al.,* 1994).

The proximate composition of meat from loin of WAD goats slaughtered at different LW is shown in Table 5. The dry matter (DM) content of goat meat across the treatments was closely related (P > 0.05). The crude protein (CP) content was significantly higher in heavier carcasses with goats slaughtered at 10kg LW having the least (P < 0.05) percentage. Goats slaughtered at 12 and 14kg LW were statistically (P > 0.05) similar in CP content while those slaughtered at 16kg LW had the highest (P < 0.05) percentage. This corroborates the reports of (Arguello *et al*., 2005) in kids slaughtered at different live weights. The effect of weight at slaughtering on the fat content revealed that fat content increases (P < 0.05) with the weight of goats. The fat content of goats slaughtered at LW of 10 and 12kg were not significantly (P > 0.05)

Table 5: Sensory properties of meat(loin) from West African Dwarf goats slaughtered at different weights

Parameters	Slaughter Weights (Kg)				
	10	12	14	16	SEM
Colour	6.67[b]	7.33[a]	7.67[a]	7.93[a]	0.04
Texture	6.17	6.67	6.33	6.71	0.08
Juiciness	5.63	5.67	5.43	5.21	0.13
Flavour	6.11[b]	6.33[b]	6.95[ab]	7.14[a]	0.16
Tenderness	6.17[b]	6.17[b]	6.67[ab]	7.47[a]	0.15
Acceptability	1.33[c]	1.83[b]	2.87[a]	2.33[a]	0.47
pH	6.08[b]	6.11[b]	6.30[a]	6.31[a]	0.12

[a,b,c] Mean values on the same row with same superscripts are not significantly different (P >0.05)
*Meat from loin

affected but were significantly (P < 0.05) lower than goats slaughtered at 14 and 16kg LW. Richetti *et al.* (1973) also reported increasing levels of intramuscular fat content with increasing body weight of goats. Also, the ash contents varied significantly (P < 0.05) among the treatments.

The variation in chemical composition of meat from WAD goats across the treatments could be attributed to age of goats at slaughtering as live weight increases with age of animals (Abanto, 1999). This corroborates earlier reports by Devendra and Owen (1983) that the chemical composition of lean tissue of meat animals is greatly influenced by body weight and goats do not seem to be an exception of this.

The influence of LW at slaughtering on the sensory properties of WAD goats are summarized. The panelists rated the colour of meat from goats slaughtered at 10kg LW to be significantly (P < 0.05) darker compared with the other treatments. This support the findings of (Arguello *et al.,* 2005) in kids of Majorera breed slaughtered at various live weights. In contrast, (Locker *et al.,*1977) reported no variation in the colour of meat from goats slaughtered at different live weights, which might be attributed to breed differences and the nutritional status of the animals coupled with the management system. In a study involving Criollo goats, it was found that as maturity increases, muscle colour significantly darkened (Pike *et al.,* 1973) which is not in consonance with the results of this study. Moreover, panelists rated texture and juiciness equal (P > 0.05) across the treatments which can be interpreted to mean that LW at slaughter does not have any influence (P > 0.05) on the texture and juiciness of meat from WAD goats. Contrary findings were reported by (Carlucci *et al.,*1998) for young goat meat which might be as a result of age, breed differences and management system. However, goats slaughtered at lower LW were observed to be more tender (P < 0.05) with a poorer flavour (P < 0.05) compared with the other treatments. Koohamaraie *et al., (*1995); Carlucci *et al., (*1998) found that a smaller area of muscle fibres, which is associated with lower weight animals, gives more tendered meat.

The preference of the panelists in this study for meat of goats slaughtered at higher LW could be as a result of the fact that most Nigerians prefer tough meat compared with the softer ones and meat tenderness has been found to be one of the most important attributes in terms of consumers' satisfaction (Arguello *et al.,* 2005). Overall acceptability was rated high (P < 0.05) by the panelists for meat

from goats slaughtered at 14 and 16kg LW, which might be attributed to the light colour and better flavour. This supports the reports of (Lapitan *et al.,* 2007) that meat consumers prefer light colour meat to darker ones as darker coloured meat may be synonymous with toughness and low quality. On the contrary, Risvik (1994) found that consumers' preferred tender and juicy meat. The variation could be attributed to the type and breed of animals used, management system and the preference of the consumers.

The pH values were significantly ($P < 0.05$) affected by LW at slaughter across the groups with higher values recorded for heavier carcasses. This supports the observations of (Marichal *et al.,* 2003) that pH values fell as LW at slaughter of kids increased from 6 to 25kg. However, the pH values after slaughter ranged from 6.08 to 6.31 and were in agreement with those found by other authors (Laskar and Nath, 1995; Arguello *et al*., 2005).

Conclusion

The haematological parameters of West African dwarf goats differed with the live weight of the animals. This result could however, serve as reference for diagnostic and therapeutic purposes in WAD goats managed intensively. Also, live weight at slaughtering had an influence on the carcass characteristics, composition and meat quality of West African dwarf goats. Goats slaughtered at 14 and 16kg LW possess high dressing percentage and their meat was most acceptable to the panelists, on account of preference for their colour, flavour and tenderness. This may serve as an incentive for butchers and could encourage the development of goat meat sector managed intensively.

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References

AMSA (American Meat Science Association) 1978. Guidelines for cookery and sensory evaluation of meat. *American Meat Science Association,* Chicago, Ilinios. pp.33.

AOAC (Association of Analytical Chemist). 1990. *Official Methods of Analysis,* 15th edition. Washington, D.C., USA, pp. 69-88.

Abanto O D, 1999. Live, carcass and meat traits of Philippine carabao, Philippine carabaro x Nlli-ravi crossbreds, graduate thesis. University of the Philippines at Los Banos, Laguna. pp. 22.

Adeloye A A, 1998. The Nigeria Small Ruminant species. *Coporate Office Max, Ilorin*, Nigeria, pp. 7-8.

Adu I F, and Brinckman, 1981. Feedlot performance and carcass characteristics of sheep fed varying concentrate levels. *Journal Animal Production Research.* 1, 1-12.

Adu I F, Aina A B J and Okeleye K A, 1996. On farm establishment and productivity of Gmelina and Gliricidia as browse for goats. *Nig. J. Anim. Prod.* 23(1): 47-52.

Arguello A, Castro N, Capote J and Solomon M, 2005. Effects of diets and live weight at slaughter on kid meat quality, *Meat Science,* 70:173-179.

Bamikole M A, Ezenwa L, Akinsoyinu A O, Arigbede M O and Babayemi O J, 2001. Performance of West African dwarf goats fed Guinea grass-verano stylo mixture, *N fertilized and Unfertilized Guinea grass. Small Ruminant Research,* 39:145-152.

Blood D C, Henderson J A and Radostists O M, 1989. *Veterinary Medicine,* 5th edition Bailliere Tindall London, pp. 866 – 926.

Carlucci A, Girolami A, Napolitano F and Monteleone E, 1998. Sensory evaluation of

young goat meat. *Meat Science*, 50 (1): 131-136.

Daramola J O, Adeloye T A, Fatoba T A and Soladoye A O, 2005. *Hematological* and *Biochemical* parameters of West African Dwarf goats. *Livestock Research for Rural Development* 17(8).

Devendra C and Owen J E, 1983. Quantitative and Qualitative aspects of meat production from goats. *World Animal Review* 47:19-28.

Duncan D B, 1955. New Multiple Range Test. *Biometrics* 11: 1- 42.

Fasae O A, Ogunmekan K O and Adu I F, 2005. The effect of palm kernel cake supplementation on the growth response and blood parameters of weaner goats fed cassava peels. In: Proceedings of the 10th Annual Conference of the Animal Science Association of Nigeria held on 12th to 15th September 2005 at the University of Ado-Ekiti, Ekiti State, Nigeria. Dairo, F.A.S., Fajemilehin, S.O.K. and Onibi, G.E. (Eds.). pp. 201-203.

Fasae O A, Emiola O S and Adu I F, 2009. Production of manure from West African dwarf sheep. Archivos de Zootenica.58 (Suppl. 1): 601-604.

Isah O A, Omorogiuwa L E and Akinnusi A O, 2007. Proximate, mineral and fibre composition of some common browse plants eaten by free roam village goats in Edo State. In: Proceedings of the 32nd Annual Conference of the Nigerian Society of Animal Production, (E.A. Agiang, E.A. Agwunobi, O.O. Olawoyin, Eds.), *University of Calabar,* Calabar, Nigeria. March 18-21, 2007, pp. 541.

Iwe M O, 2002. Handbook of sensory method of analysis. Rejoint communications services Ltd. 1st edition, pp. 70-71.

Lapitan R M, Del Barrio N D, Katsube O, Ban-Tokuda T, Orden E A, Cruz L C, Homma H and Kanni Y, 2007. Comparison of carcass and meat characteristics of Brahman grade cattle and crossbred water buffalo. *Animal Science Journal* (78): 596-604.

Laskar S K and Nath D R, 1998. Studies on certain qualitative aspects of goat meat marketed in Guwahati city. *Indian Journal of Management*, 14:217.

Locker R H, Davies G L, Carse W A and Leet N G, 1977. Meat tenderness and gap filament. *Meat Science* 1:81-104.

Marichal A, Castro N, Capote J, Zamorano M J and Argüello A. 2003. Effects of live weight at slaughter (6, 10 and 25 kg) on kid carcass and meat quality. *Livestock Production Science* 83(2-3): 247-256.

Ockerman H W, 1985. Quality control of post mortem muscle tissue, 13th edition, The Ohio University, Colombus, OH.

Oni O O, 2002. Breeds and Genetic improvement of small ruminants. In: Small Ruminant Production Training Workshop, National Animal Production Research Institute, Shika, Zaria. 13th – 18th January, 2002, pp. 1-7.

Orheruata A M, Osueni J E and Aperua-Yusuf A O, 2004. Studies on haematological indices of West African Dwarf goats in different location in Edo State, Nigeria. *Asset Series.* A 4(1): 1 – 7.

Pike M I, Smith G C, Carpenter Z L and Shelton M, 1973. Effects of maturity and fatness on the palatability of goat meat. *Journal of Animal Science* 37: 269.

Puls R, 1994. Mineral levels in animal health: diagnostic data. *Normal levels summary sheets,* pp.302-308.

Richetti F, Intrieri F, Fabiano N and Franciscus G, 1973. Research into the chemo bromatological characteristics of goat kids slaughtered at 10 days. *Atti della Societa Italiana della Scienze Vet.* 27: 439-497.

Risvik E, 1994. Sensory properties and preferences. *Meat Science,* 36: 67-77.

SAS (Statistical Analysis system) 1999. SAS User's guide: SAS Institute Inc., Cary North Carolina, USA.

Singh L B, Singh D K and Singh C S P, 1994. Genetic studies on the carcass characteristics of black Bengal goats. *Indian Journal Animal Science*

64(2): 157-162.

Tambuwal F M, Agaie B M and Bangana B, 2002. *Haematological* and *biochemical* values of apparently healthy red sokoto goats. In: Proceedings of the 27th Annual Conference, *Nigerian Society for Animal Production,* March 17-21, 2002. Federal University of Technology, Akure, Nigeria, pp. 50-53.

Webb E C, Lasey N H and Simela L, 2005. Goat meat quality. *Small Ruminant Research*, 60: 153-156.

Effect of hemiorchidectomy on the growth performance, linear body and testis measurements of boars in the humid tropics

Effet de l'hémiorchidectomie sur la croissance, les mesures des formes linéaires du corps et des testicules des sangliers des régions tropicales humides

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Abstract

An experiment was conducted to determine the impact of hemiorchidectomy on growth performance, linear body and testis measurements of weaner boars. A total of 18, ten-weeks old crossbred Landrace x Large White boars with average body weight of 13.01±1.32kg were randomly assigned to two treatment groups (T1-*hemi-orchidectomised boars*; and T2-uncastrated boars). The experimental boars were given 18% crude protein and 4.96Kcal ME/kg diet. Daily feed intake, daily weight gain, final body weight and protein intake of the boars were significantly ($P<0.05$) higher for uncastrated intact boars. Feed conversion ratio and protein efficiency ratio between hemi-orchidectomised and uncastrated intact boars were similar ($P>0.05$). *Hemiorchidectomy* significantly ($P<0.01$) affected the heart girth and testis width measurements of the boars. Heart girth measurements was significantly ($P<0.05$) higher in the uncastrated intact boar whereas, testis width measurements were significantly ($P<0.01$) higher in the hemiorchidectomised boars. Correlation among linear body and testis measurements and body weight of hemi-orchidectomised and uncastrated intact boars were positive and highly significant ranging from 0.83 to 0.96 for the boars on T1 and 0.70 to 0.96 for boars on T2. There was evidence of compensatory testicular growth in the remaining testis after hemiorchidectomy. However, since hemiorchidectomy of boars in this study did not appear to improve their growth performances, its application and use in animal production should be discouraged. It was concluded that hemiorchidectomy should not be practiced in any pig production enterprise since significant improvements in the growth performance of the affected boars were not achieved.

Key words: Pig, *Hemiorchidectomy, Testis*, boar, *Compensatory Testicular* growth,

Résumé

La détermination de l'impact de l'hémiorchidectomie sur la croissance, sur les mesures des formes linéaires du corps et sur les mesures de testicules de sangliers sevrés a fait l'objet des travaux de recherche . Un total de 18 sangliers croisés landrace x large white âgés de 10 semaines (70 jours) avec un poids corporel moyen de 13,01 ± 1,32 kg choisis de maniéu alléatoire, ont été soumis à deux groupes de traitement (T1-sangliers ayant subi l'hémiorchidectomie et T2 sangliers non castrés). Les sangliers sélectionnés pour l'expérience ont été soumis à un régime nutritionnel de 18% de protéines brutes et 4.96Kcal ME par kg d'aliment. La consommation quotidienne d'aliment, le gain de poids quotidien, le poids corporel final et l'ingestion en protéines des sangliers ont été sensiblement plus élevés ($P<0,05$) pour les sangliers non castrés restés intacts. L'indice de conversion de la alimentaire et le coefficient d'efficacité protéique entre les sangliers ayant subi l'hémiorchidectomie et les

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sangliers non castrés restés intacts étaient similaires (P>0,05). L'hémiorchidectomie a sensiblement affecté les mesures (P<0,01) du périmètre thoracique des sangliers non castrés restés intacts (P <0,05), de même que les mesures de la largeur des testicules (P <0,01) se sont avérées sensiblement supérieures à celles des sangliers hémiorchidectomisés. La corrélation entre les mesures linéaires du corps et les mesures des testicules et le poids corporel des sangliers hémiorchidectomisés non castrés et restés intacts a été positive et très significative, et variait de 0,83 à 0,96 pour le groupe T1 des sangliers hémiorchiectomisés, et de 0,70 à 0,96 pour les sangliers appartenant au groupe T2 des sangliers non castrés. L'on a décelé des preuves de croissance testiculaire compensatoire dans le testicule restant après l'opération d'hémiorchidectomie. Toutefois, à partir du moment où, selon les études effectuées, l'opération d'hémiorchidectomie de sangliers n'aboutit pas à l'amélioration de leur croissance, son application et son utilisation dans la production animale devrait être découragée. La conclusion à tirer de cette expérience est que l'hémiorchidectomie devrait être bannie de toute entreprise de production porcine en raison du fait qu'elle ne joue aucun rôle dans l'amélioration de la croissance des sangliers soumis à cette pratique.

Mots clés: Porc ; hémiorchidectomie ; testicules ; sanglier ; croissance du testicule ; compensatoire

Introduction

Hemiorchidectomy, which is a surgical removal of one testis, is now a common practice in studies involving compensatory *hypertrophy* of the testis in males of various mammalian species (Lunstra *et al*., 2003, Tesfaye *et al*., 2008). In ruminants (Barnes *et al.,* 1980) and in boars (Minton and Wetteman, 1982) it was been reported to cause hypertrophy and increase in the weight of the retained testis. Onuora and Omeke, (1989) and Cunningham, *et al.,* (1978) further reported that the *hypertrophy* of the remaining testis following hemi-castration was age-dependent in pigs. Umesiobi (2006) also reported that the degree of compensatory testicular *hypertrophy* may serve as an indicator of the reproductive status of the boar. Many reports in the literature have appeared on the effect of hemi-castration on the compensatory hypertrophy of the remaining testis and sperm production. According to Edgren *et al.* (1965); Jenkins and Waites (1983) and Onuora and Omeke (1989), the processes involved in the compensatory hypertrophy of the remaining testis is associated with negative feedback mechanism between inhibin and the pituitary gland causing increase in the prepubertal plasma gonadotropin levels. Few reports are available on the effect of hemiorchidectomy on growth performance and linear body measurements of pigs in the tropics. Our hypothesis was that surgical method of achieving hemiorchidectomy in pigs is a form of stress and could possibly hinder the overall growth performance and linear body and testis measurement indices of the animal. This study, therefore, was designed to determining the effect of hemiorchidectomy on growth performance, linear body and testis measurements of weaner pigs raised in a tropical humid environment.

Materials and Methods

The experiment was conducted at the piggery unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. A total of eighteen ten week (70 day) old crossbred Landrace x Large White weaner boars selected from experimental pigs managed in the farm were used for the study. Average body weight of the boars was 13.01 ± 1.32kg. The boars were randomly assigned to two treatment groups namely treatment 1 (T1 – hemi-orchidectomised *boars*) and treatment 2 (T2 – uncastrated control boars) with each having nine animals divided into three groups which served as replicates. The boars in T1 were subjected to hemiorchidectomy involving the surgical

Table 1: Percentage Composition of Experimental Diet

Ingredient	Percentage Composition (%)
Maize	25.80
Wheat Offal	19.35
Cassava Peel	19.35
Palm kernel cake	8.55
Groundnut cake	11.40
Soya bean meal	5.70
Fish meal	2.85
Salt	0.25
Premix	0.25
Bone meal	3.5
Oyster shell	2.5
Lysine	0.25
Methionine	0.25
Total	**100**
Calculated	
Crude protein (%)	18.04
Metabolizable Energy (KcalME/kg)	4.96
Crude fibre (%)	10.59

Table 2: Proximate and Energy Composition of the Experiment Diet

Composition (Dm, Basis)	Experimental Diet (%)
Crude Protein	18.04
Ether Extract	0.15
Crude Fibre	10.60
Ash	11.25
Moisture	07.25
Energy Kcal ME/kg	5.03

removal of the left testis on first day of the experiment. *Hemiorchidectomy* was done by exposing one testis through unilateral proximal scrotal incisions and surgical removal (Umesiobi, 2006). Procedures for surgical incision and removal of the testis were in line with approved guideline for *humane* treatment of animals (FASS, 1999). The testes of boars in T2 were left intact throughout the 12 weeks duration of the study. The boars were each housed in pens with concrete floor measuring 2m x 3m each. The windows of the pig house were fitted with fly-proof nets to prevent flies from entering the pens. Water was provided *ad libitum* whereas feeding was done twice daily at 0800 and 1400hrs. Boars were scale-fed according to their body weight (6% of body weight). Cleaning of the pens was done daily before feeding the animals. The experimental diet used contained 18% CP and 4.96 KcalME/kg diet. The percentage and proximate composition of the diet are shown in Tables 1 and 2, respectively. The boars were weighed weekly to determine body weight while linear body and testis measurements were also taken on each animal immediately after weighing. This was done between 07.30h before morning feeding and 10.00h. The linear body and testis measurements taken were heart girth (HG), body length (BL), flank-to-flank (FF), testis length, and testis diameter. Procedure for linear body measurements were in line with the report of Machebe and Ezekwe (2010). Measurements for live weight (LW) and other linear body and testis measurements were taken three times after which an average was taken as a representative figure. A total of 648 measurements were recorded. Other performance parameters determined were daily feed intake, daily weight gain, feed conversion ratio, protein intake, and protein efficiency ratio.

Statistical Analysis

The means and standard error of body weight (initial and final), daily feed intake, daily weight gain, protein intake, protein efficiency ratio and body and *testicular* measurements of boars in the two experimental treatment groups were computed using SAS (1999) computer package. Means for the various parameters measured were subjected to a two-tailed T-test statistics at 5% level of probability to determine significant differences. Relationships between body weight and other body and testis measurements were determined by Pearson correlation method.

Table 3: Growth performance of *hemi-orchidectomised* and normal intact weaner boars

Parameters	Treatments		Probability level
	Hemi-orchidectomised boars	Uncastrated boars (control)	
Initial Body Weight (kg)	11.24± 3.25	12.33 ± 1.45	P=0.05
Final Body Weight (kg)	34.57±2.96b	39.83±4.89a	P=0.04*
Daily feed intake (kg)	1.27±0.07b	1.48±0.10a	P=0.05*
Daily weight gain (kg)	0.26±0.12b	0.33±0.11a	P=0.02*
Feed conversion ratio	4.34±8.26	5.27±2.88	P=0.53NS
Protein intake (kg)	0.24±0.01b	0.28±0.02a	P=0.05*
Protein efficiency ratio	1.09±0.44	1.24±0.45	P=0.15NS

a,b – row means with different superscripts are significantly different at 5% (*- P<0.05); NS = Not-Significant

Table 4: Linear body and testis measurements of *hemi-orchidectomised* and normal intact weaner boars

Parameters	Treatments		Probability level
	Hemi-orchidectomisd boar	Uncastrated boars (control)	
Body Length	75.36 ± 1.70	78.44± 1.90	P=0.06NS
Heart girth	57.88 ± 1.25b	62.45 ± 1.06a	P=0.03*
Flank to flank	52.92± 1.34	54.09 ± 1.34	P=0.12 NS
Testis length	11.88 ± 0.73	11.16 ± 0.61	P=0.59 NS
Testis width	7.23 ± 0.51a	5.62±0.26b	P=0.001**

[a,b] – row means with different superscripts are significantly different at 1% (**-P<0.01) or 5% (*- P<0.05); NS = Not-Significant

Results

Result of the effect of hem-castration on growth performance of weaner boars are presented in Table 3. Final body weight, daily feed intake, daily weight gain and protein intake were significantly (P<0.05) lower in hemi-orchidectomised boars when compared with normal boars. Feed conversion ratio, and protein efficiency ratio of hemi-orchidectomised and normal boars were similar (P>0.05). *Hemiorchidectomy* of the boars affected heart girth and testis width measurements. The heart girth of *hemi-orchidectomised* boar were significantly (P<0.05) lower when compared with that of the normal boar (57.88cm Vs 62.45cm, respectively). On the other hand, testis width measurements of hemi-orchidectomised *boars* (7.23cm) were highly significantly (P<0.01) different from values recorded for normal boars (5.62cm). No significant differences (P>0.05) in body length, flank-to-flank and testis length measurements were obtained between hemi-orchidectomised and normal boars. Weekly variations in body weight, body length, heart girth, flank-to-flank, testis length and testis diameter measurements between hemi-orchidectomised and normal boars are shown in Figures 1 and 2, respectively.

Correlation among linear body and testis measurements and body weights for hemi-orchidectomised *boars* are presented in Table 5. *Correlation* among the traits ranged from 0.83 to 0.96. The efficiency of measurements (r^2) among the various parameters ranged from 0.71 to 0.92. Body weight measurements of the boars were significantly correlated with testis width and testis length measurements (r=0.91 and 0.93, respectively). *Correlation* between

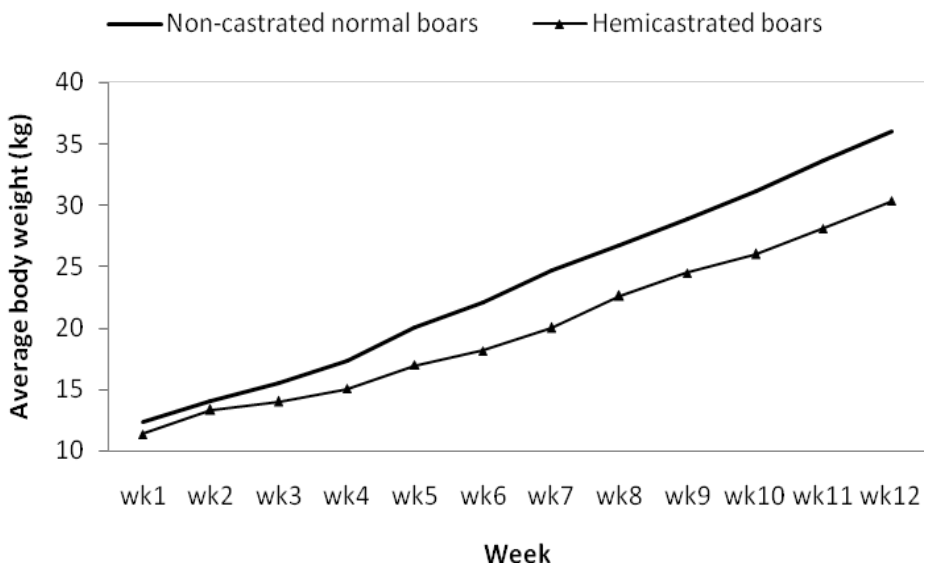


Fig 1: Weekly Variation in the body weight of Hemi-castrated and Non-castrated normal boars

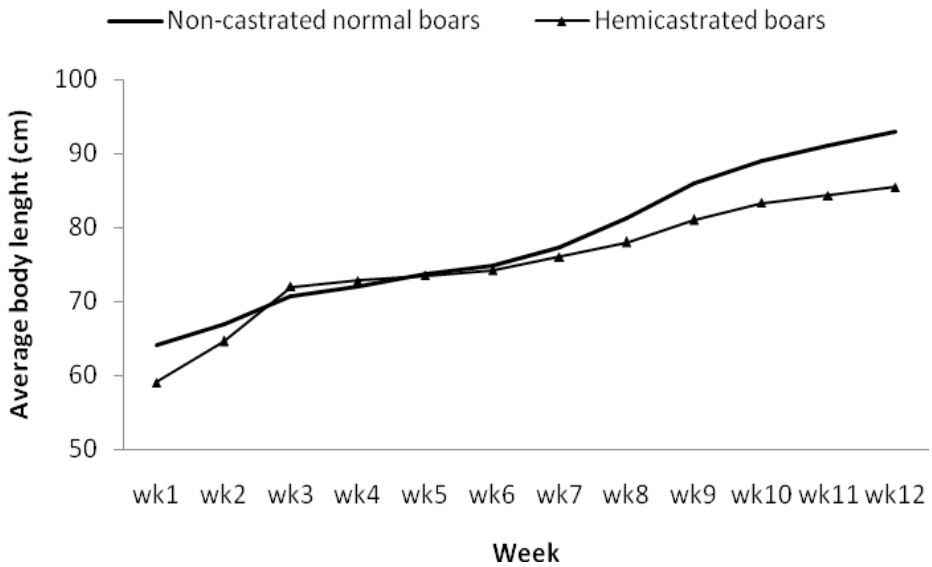


Fig. 2: Weekly variation in the body length measurement of Hemi-castrated and Non-castrated normal boars

testis measurements and other linear body measurements was less than 90%. A very highly significant (P<0.001) *correlation* coefficient of 0.95 (r^2=0.90) was obtained between testis diameter and testis length measurements.

Correlation between linear body and testis measurements and body weight of normal boars was highly significant (P<0.01) (Table 6). Correlation efficient among linear body and testis measurements and body weight ranged from 0.70 to 0.96. The efficiency of measurement (r^2) also ranged from 0.49 to 0.95. The correlation coefficient between body weight and body length (0.95), heart girth (0.94), flank-to-flank (0.94), testis length (0.79) and testis width (0.71) were highly significant (P<0.01). In addition, correlation coefficient between testis length and testis diameter was strong and highly significant (r=0.85; P<0.01).

Table 5: Correlation coefficient between body weight and some linear body and *testis* measurement of *hemicastrated* boars

Parameters	Body weight(kg)	Body length (cm)	Heart girth (cm)	Flank to flank (cm)	Testis length (cm)	Testis width (cm)
Testis diameter (cm)	0.91** (0.83)	0.84** (0.71)	0.89** (0.79	0.84** (0.71)	0.95** (0.90	-
Testis length (cm)	0.93** (0.86)	0.85** (0.72)	0.89** (0.79)	0.88** (0.77)	-	
Flank-to-flank (cm)	0.96** (0.92)	0.87** (0.76)	0.89** (0.79)	-		
Heart girth (cm)	0.96** (0.92)	0.95** (0.90)	-			
Body length (cm0	0.91** (0.83	-				
Body weight (kg)	-					

(** - P<0.01);Values in parenthesis represents R2 (Efficiency of measurement)

Table 6: Correlation coefficient between body weight and some linear body and testis measurement of noncastrated normal boars

Parameters	Body weight(kg)	Body length (cm)	Heart girth (cm)	Flank to flank (cm)	Testis length (cm)	Testis width (cm)
Testis diameter (cm)	0.71** (0.50)	0.71** (0.50)	0.70** (0.49)	0.78** (0.61)	0.85** (0.72)	-
Testis length (cm)	0.79** (0.62)	0.79** (0.62)	0.77** (0.59	0.81** (0.66)	-	
Flank-to-flank (cm)	0.94** (0.88)	0.96** (0.92)	0.97** (0.95)	-		
Heart girth (cm)	0.94** (0.88)	0.96** (0.92)	-			
Body length (cm0	0.95** (0.90)	-				
Body weight (kg)	-					

(** - P<0.01);Values in *parenthesis* represents R2 (Efficiency of measurement)

Discussion

The significant decrease in final body weight, daily feed intake, daily weight gain and protein intake observed in hemi-orchidectomized *boars* as against the normal boars may have occurred due to stress experienced during orchidectomy. According to Hopkins-Shoemaker *et al.* (2004) reported that castration as a management practice imposes unnecessary pain and stress to animals. Animals subjected to castration (hemi- or bilateral orchidectomy) undergoes several weeks of anxiety, stress and aggression (Thun *et al*., 2006) this could have caused a decrease in appetite and reduction in feed and protein intakes of the boars observed in this study. Reductions in daily weight gain of the hemi-orchidectomised boars as compared with the normal boars have been reported (Sundby *et al*., 1981; Odo, 2003). Sundby *et al*. (1981) reported that hemi-castration did not appear to have any significant effect on

growth rate in boars. However, the authors noted that castration per se have a slight effect on testicular steroids in plasma in pigs at 5-7 months of age. The reduced weight gain of the hemi-orchidectomised boars during the period of the study may be linked with the overall reduction in secretion of testosterone caused by the removal of one testis during orchidectomy. Testosterone, a male hormone secreted by the *testis* has an anabolic growth effect and a strong relationship have been reported to exist between testosterone and muscle growth (Arnold *et al.,* 1997). It may be inferred that high production of testosterone in the paired testis of intact male boars may have accounted for the higher significant growth performance in the control intact group of boars. In support, Onuora and Omeke (1989) reported that the extent of testicular compensatory hypertrophy and the concomitant plasma concentration of androgen between hemi-castrated animals and the intact animals may explain the presence of significant differences between the body weights. On the contrary, Balaji *et al.* (2006) did not record any significant difference ($P>0.05$) in the daily body weight gain between castrated and uncastrated Large White Yorkshire pigs. The authors noted that uncastrated boars had significantly ($P<0.01$) better feed efficiency compared with castrated boars. Age of castration may have influenced the growth performance of the boars due to the physiological effect of hemiorchidectomy on boars. According to Onuora and Omeke (1989), the age at which hemi-castration was carried out in piglets influenced their growth rate. Boars hemi-orchidectomised at one month of age had a maximum increase in testis weight compared with boars hemicastrated at 3, 4, 5, 6, and 7 months of age (Putra and Blackshaw, 1985). Possible variations in growth performance of hemi-orchidectomised or *bilateral orchidectomised* boars reported may occur due to breed differences, feeding management, age and weight at castration and method of castration used (Tesfaye *et al.,* 2008) and experimental period.

The highly significant ($P<0.01$) increase in testis width measurement of hemi-orchidectomised boars was not surprising and could be attributed to compensatory growth of the remaining testis after hemiorchidectomy. This agrees with the observations of Sundby *et al.,* (1981), Minton and Wetteman (1982), Onuora and Omeke (1989), and Lunstra et al (2003) in boars. Orth (1993) reported that unilateral castration of prepubertal males causes compensatory hypertrophy and increased size in the remaining testis, but hypertrophy generally occurs only if unilateral castration is performed before puberty. According to Lunstra *et al.* (2003), hemiorchidectomy of prepubertal boars lead to an increase in the mass of the remaining testis. The hypertrophy and increased size of the remaining testis has been associated with increased diameter and length of the seminiferous tubules, increased numbers of germ and Sertoli cells and increased sperm production per testis (Orth, 1993). Available literature (McCoard *et al*., 2001; Lunstra *et al*., 2003) have indicated that in most species, normal testicular growth is associated with dramatic proliferation of Leydig cells in the interstinium and an increased number of Sertoli and germ cells within the seminiferous epithelium. General compensatory hypertrophy effects have

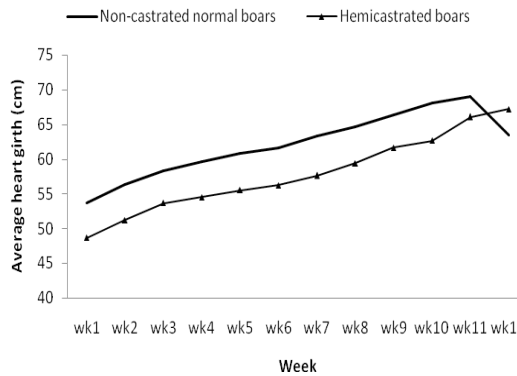


Fig. 3: Weekly variation in the heart girth measurements of Hemi-castrated and Non-castrated normal boars

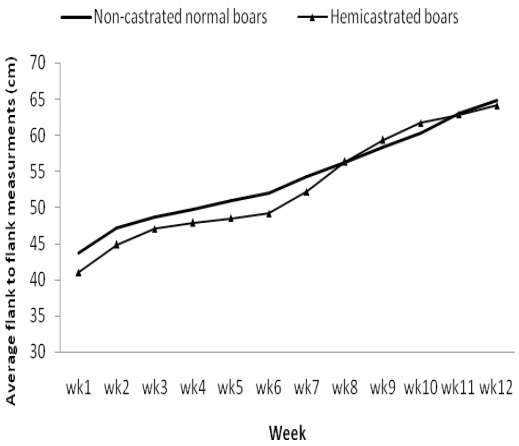


Fig. 4: Weekly variation in the flank-to-flank measurements of Hemi-castrated and Non-castrated normal boars

been reported in boars (Waites *et al.,* 1983; Kosco *et al.,* 1989; Umesiobi 2000; Lunstra *et al.*, 2003). A general increase in the testicular sperm compactments (tubules and interstitium) and an increase in the total number of Sertoli cells usually occur when boars are orchidectomised at a younger age (Lunstra *et al.,* 2003). Compensatory hypertrophy of the remaining testis in hemi-orchidectomised *boars* has been linked to increased plasma follicle stimulating hormone (FSH). Ramaswamy *et al.* (2000) reported that pubertal boars exhibited prolonged elevation of plasma FSH after hemi-castration and that FSH appeared to also modulate the increased sperm production observed in hemi-castrated *boars* via a stimulatory effect on differentiated spermatogonia. An elevated level of FSH has been reported to occur immediately after castration (Ford *et al.*, 2001). A closer observation of Fig. 5 showed that although there was a non-significant (P>0.05) increase in testis length between the hemi-orchidectomised and normal boars, there was tendency for marked variation in testis length measurements of the remaining testis to occur 8 weeks (about 56 days) after castration. On the other hand, a sharp and highly significant (P<0.01) increase in testis width measurements of the remaining testis in hemi-orchidectomised boar begins a few days (about 14-21day) after castration (Fig.6). Weekly variation in body length, heart girth, flank-to-flank measurements for the two group of boars did not vary (P>0.05) (Figures 2, 3, and 4).

Correlation coefficients between body weight and other linear body and testis measurement indicates that a much stronger and reliable relationship exist among the traits measured in hemi-orchidectomised group of boars than in their normal counterpart. Thus, it is possible that in hemi-orchidectomised boars, linear body measurements (Body length, heart girth and flank-to-flank) and *testis* (testis length and testis width) measurements could serve as a tool for prediction of body weight in *hemi-orchidectomised* boar. Although there was a significant relationship between body weight and the linear body and testis measurements in the normal group of boars, only flank-to-flank (r=0.96; r^2=92%), heart girth (r=0.96; r^2=92%), and body length (r=0.95; r^2=90%) measurements could also be used to predict body weight of normal intact boars. The use of these linear body measurements for prediction of body weight of gilts had been documented (Machebe and Ezekwe, 2010). A very strong and reliable relationship exists between testis length and testis width. However, the relationship between the two testis traits was higher in hemi-orchidectomised boar (r=0.95; r^2=90%) than normal boars (r=0.85; r^2=72%), suggesting a more proportional increase in the testis length and width in hemi-orchidectomised boars than in normal intact boars. A similar strong and positive relationship (r=0.71; r2 =0.49) between testis length and testis width have been reported.

Conclusion

From the results of the study, pre-pubertal hemi-orchidectomy induces compensatory testicular hypertrophy of the remaining of testis boars. However, it should not be practiced in any commercial pig production enterprise since significant

improvements in the growth performance of the affected boars were not achieved.

Impact

Hemiorchidectomy has been used as a model for studying factors influencing testicular development in mammals. It has been applied in studies to an understand of the mechanisms responsible for increases in testis length and width of the remaining testis after hemicastration. This study contributes to this knowledge. The study, however, compaired with the welfare of the *hemicastrated* animal. It indicated that the surgical removal of one testis affect the *physiology* of the animal because of pain and discomfort to the animal and thus, leading to stress. Physiological stress caused by hemicastration affects the overall growth performance of the animal. It was suggested that except in studies, unilateral removal of one testis in boars should not be practices in any commercial pig enterprise.

Acknowledgement

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References

Arnold A M, Peralta J M and Yhonney M L. 1997. Effect of *testosterone* on differential muscle growth and on protein and nucleic acid concentration in muscles of growing lambs. *Journal Animal Science,* 75:1495-1503.

Balaji N, Sri Sivaraman T, Sivakumar T and Ramesh V. 2006. Influence of castration on growth rate and body measurements in Large White Yorkshire pigs. *Indian Journal of Animal Research,* 40(2):

Barnes N A, Longnecker J V, Charter R C, Riessen J E and Woody C O. 1980. Influence of unilateral castration and increased plane of nutrition on sexual development of Holstein bulls.1. *Growth and sperm production. Theriogenology,* 14: 49-58.

Cunningham G R, Tindall D J, Huckins C and Means A R, 1978. Mechanisms for the testicular hypertrophy which follows *hemicastration. Endocrinology* 102(1): 16-23.

Edgren R A, Parlow A F, Peterson D L and Jones R C. 1965. Mechanism of *ovarian hypertrophy* which follows *hemicastration. Endocrinology*, 76:97-102.

Hopkins-Shoemaker C, Solaiman S, Keith C, Jones W and Bransby D. 2004. Growth and carcass characteristics of castrated or intact male Boer x Spanish goats grazing marshall annual ryegrass. *Journal of Animal Science,* (82) suppl. 1

FASS, 1999. Guideline for the Care and Use of Agricultural Animal in Agricultural Research and Teaching. 1st rev. ed. Savoy, *Il: Federation of Animal Sciences*. Pp.313-412.

Ford J J, Wise T H, Lunstra D D and Rohrer G A. 2001. Interrelationships of *porcine* X and Y *chromosomes* with pituitary gonadotropins and testicular size. *Biology of Reproduction*, 65:906-912.

Jenkins N and Waites G M N. 1983. Effect of *hemicastration* of various ages and of oestradiol 17β on plasma concentration of *gonadotrophins* and androgens on testicular growth and interstitial cell responses in prepubertal lambs. *Journal of Reproduction and Fertility*, 67:325-334.

Kosco M S, Loseth K J and Crabo B G, 1989. Development of the *seminiferous tubules* after neonatal hemicastration in the boar. *Journal of Reproduction and Fertility* 87:1-11.

Lunstra D D, Wise T H and Ford J J. 2003. Sertoli cells in the Boar Testis: Changes during Development and Compensatory *Hypertrophy* after *Hemicastration* at Different ages. *Biology of Reproduction* 68:140-150.

Machebe N S and Ezekwe A G. 2010. Predicting body weight of growing-finishing gilts raised in the tropics using linear body measurements. *Asian Journal of Experimental Biological Science,* 1(1) :162-165

McCoard S A, Lunstra D D, Wise T H, and Ford J J, 2001. Specific staining of Sertoli cell nuclei and evaluation of Sertoli cell number and proliferation activity in Meishan and White Composite boars during the neonatal period. *Biology of Reproduction,* 64:689-695.

Minton J E and Wetteman R P, 1982. The influence of photoperiod and *hemicastration* on growth rate and testicular functions in young boars. *Journal of Animal Science,* 55(Suppl. 1): 42-43.

Odo B I. 2003. Note on the effect of hemicastration on growth parameters in pigs. *Livestock Production Science,* 82 (2-3): 255-258.

Onuora G I and Omeke B C O. 1989. Effect of Age at Hemicastration on the Body and Testis Weight of Piglets. *Animal Reproduction Science*, 18: 105-110

Orth J M. 1993. Cell biology of testicular development in *fetus* and *neonate*. In: Desjardins C, Ewing L.L. (Eds), cell and molecular biology of the testis, 1st Ed. *New York, Oxford University Press;* pp 3-42.

Putra D K and Blackshaw A W. 1985. Quantitative studies of compensatory testicular *hypertrophy* following unilateral castration in the boar. *Australian Journal of Biological Science,* 38:429-434.

Ramaswamy S, Plant T M & Marshall G R 2000 Pulsatile stimulation with recombinant single chain human *luteinizing hormone* elicits precocious *Sertoli* cell proliferation in the juvenile male rhesus monkey (Macaca mulatta). *Biology of Reproduction* 63 82–88.

SAS Institute. 1999. SAS/STAT. User's guide version 8 for windows. SAS Institute Inc. Cary, NC, USA Schinckel, A.P; Johnson R.K and Kittok R.J. 1984. Relationship among measures of testicular development and endocrine function in boars. *Journal of Animal Science*, 58 (5): 1255-1261.

Sundby A; Andresen O, and Standal N., 1981. Effect of *hemicastration* on the level of testicular steroids and growth in bulls and boars. *Theriogenology* 16:249-257.

Tesfaye K, Tesfaye L, Hunduma D, Mieso G and Amsalu S. 2008. Growth performance and carcass characteristics of Arsi-Bale castrated at different ages. *World Applied Sciences Journal* 4 (4):545-553.

Thun R, Gajewski Z and Janett F. 2006. Castration in male pigs: Techniques and animal welfare issues. *Journal of Physiology and Pharmacology. Supp.* 8:189-194

Umesiobi D O, 2000. Animal Production I. Basic Principles and Practices. *Bean Blaise Publication, Owerri, Nigeria*. pp.111-129.

Umesiobi D O, 2006. The effect of *hemiorchidectomy* on reproductive traits of boars. *South African Journal of Animal Science* 36(3):181-188.

Waites G M H, Wenstrom J C, Crabo BG and Hamilton D W, 1983. Rapid compensatory *hypertrophy* of the lamb testis after neonatal *hemiorchidectomy*: endocrine and light *microscopial morphometric* analysis. *Endocrinology* 115:1248-1255

EFFECTS ON PERFORMANCE OF GROWING PIGS FED DIETS CONTAINING DIFFERENT LEVELS OF CASHEW NUT REJECT MEAL.

EFFETS, SUR LES PORCINS EN PÉRIODE DE CROISSANCE, DES RÉGIMES ALIMENTAIRES À BASE DE DIFFÉRENTS NIVEAUX DE RÉSIDUS DE NOIX DE CAJOU

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Abstract

The purpose of this study was to investigate the use of reject cashew kernels (RCK), as a feed ingredient in growing pig diets. The growth rate, feed intake, feed to gain ratio and cost of gain of growing pigs fed diets containing 0 (0RCK), 150 (150RCK), 150 (150DrCP) or 300 (300RCK) gkg-1 of reject cashew kernel was investigated in a feeding trial set up as a completely randomized design (CRD) with 3 treatments replicated 5 times and lasting 140 days. There were no significant differences (P > 0.05) among treatments in the parameters measured, except for cost of gain. Cost of gain was significantly different among the three treatments (P < 0.05) with T30 being the most efficient (Ghana cedis1.59) followed by T15 (Ghana cedis 2.08) and then T0 (Ghana cedis 2.54). Overall mean growth rate, feed intake and feed to gain ration were 0.38 ± 0.008 gkg-1, 2.23 ± 0.047 kg day-1 and 5.79 ± 0.195, respectively. It was concluded that reject cashew kernels could be used in growing pig diets up to a level of 300 gkg-1 without any deleterious effects and is actually more economical. Future work will look at higher levels of RCK inclusion in the diets of growing pigs.

Key Words: Cashew, reject cashew kernels, growing pigs, feeding trial.

Résumé

La présente étude se propose d'établir les conséquences de l'utilisation des résidus de noix de cajou (RNC), comme composants alimentaires dans la nutrition des porcs en période de croissance. Le taux de croissance, la consommation alimentaire, l'indice de consommation par rapport au gain et le coût du gain des régimes de nutrition des porcs en croissance contenant 0 (0 RNC), 150 (150 RNC), 150 (150 DrCP) ou 300 (300 RNC) g kg-1 de résidus de noix de cajou ont fait l'objet de travaux de recherche dans le cadre d'une expérience conçue sur une base complètement aléatoire de 3 traitements répétés 5 fois et censés durer jusqu'à 140 jours. Il ny avait pas de différences significatives de paramètres mesurés (P> 0,05) entre les traitements, à l'exception du coût de gain. Le coût de gain a été sensiblement différent entre les trois traitements (P <0,05). Le T30, s'est avéré le plus efficace (GH¢1,59), immédiatement suivi du T15 (GH¢2,08), et enfin le T0 (GH¢2,54). Le taux global de croissance moyen, la consommation alimentaire et l'indice de consommation par rapport au gain étaient de 0,38 ± 0,008 g kg-1, 2,23 ± 0.047 kg par jour-1 respectivement. L'on est parvenu à la conclusion que les résidus de noix de cajou peuvent être utilisés dans la nutrition des porcs pendant leur période de croissance jusqu'au niveau de 300 g kg-1, sans aucun effet délétère et que ce régime alimentaire est en fait plus économique. Les travaux futurs se pencheront sur des niveaux plus élevés de l'nclusion des résidus de noix de cajou dans l'alimentation des porcs en croissance.

Mots clés: Noix de cajou ; résidus des noix de cajou ; porcs en croissance ; expérience de nutrition

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Introduction

Increase in prices of conventional feed ingredients continues to be a problem for pig farmers and has increase the search for local agro-industrial by-products which can be used as alternatives (Barnes *et al.,* 1985; Okai and Bonsi, 1989; Rhule, 1995; Adomako *et al.,* 1999; Bruce *et al.,* 2004; Attoh-Kotoku *et al.,* 2007; Rhule *et al.,* 2007 and Ayivi, 2008). The development of cashew (*Anacardium occidentale*) as an important cash crop in Ghana has led to the local processing of cashew nuts into kernels. The process generates some kernels which are discarded because they are not suitable for sale as a result of being broken or oily, or because they are scorched during the drying process. It is estimated that up to about 30% of kernels may be lost in this manner depending on the quality of nuts. A lot depends on factors that are beyond the control of the processor such as drying of nuts, which if not done properly will produce several oily nuts (Dickson Twum, *Personal communication*). Fetuga *et al.* (1975) and Fanimo *et al.* (2004) both reported an appreciable nutrient content for reject (unmarketable) cashew kernels (Fetuga et al., 1975) 216 gkg-1 crude protein, 455 gkg-1 ether extract and 30.22 MJ kg-1 gross energy; (Fanimo *et al., 2004*) 215 gkg-1 crude protein, 455 gkg-1 ether extract and 30.33 MJ kg-1 gross energy).

Fetuga *et al.,* (1975) and Fanimo *et al.,* (2004) also reported that reject cashew kernels gave good results when used in growing pig diets at a rate of 200 g/kg and 100 g/kg, respectively. Although there is not much information on the use of unmarketable cashew kernels in animal feeding, similar oil seeds such as groundnut and soya beans are used in the animal feed industry. The oil in these seeds is, however, extracted for other uses before the residue is used as animal feed

This trial was therefore carried out to investigate the use of reject cashew kernels as an ingredient in diets for growing pig.

Materials and Methods

The trial was conducted at the Worakese Plantation of the Cocoa Research Institute of Ghana. Fifteen (15) Large White growing pigs were used for the experiment and were fed for 112 days. There was an initial adjustment phase of 10 days to allow pigs to adjust to their new diet. Pigs were housed individually. Pens were roofed (aluminium) and were constructed with cement blocks and had concrete floors with a rough finish. Pigs were balanced for age, sex and litter and randomly allocated to one of three treatments having 0 (0 RCK), The experiment was laid out as a completely randomized design with three (3) treatments and five (5) replicates making a total of 15 experimental units. 150 gKg-1 (150 RCK), and 300 gKg-1 (300 RCK) reject cashew kernels, respectively. A price of Ghana cedis 0.12 was assigned to dried cocoa pod husk based on the price of wheat bran and the nutrient contents of dried cocoa pod husk relative to wheat bran. Reject cashew kernels were purchased from a cashew processing factory at Mim, in the Brong Ahafo region, and cost Ghana cedis 0.40 per kilogram. The reject cashew kernels were sub-sampled and analysed (AOAC, 2000) and the results used in feed formulation. Diets were formulated to be iso-energetic and iso-nitrogenous and were based on standard growing pig diets (See Table 2).

Pigs were fed the diets, equivalent to 5% of their body weight, once a day and water was provided *ad libitum.* Any feed left over at the beginning of the next day was weighed and subtracted from that which had been fed the previous day to determine feed intake. Feed allowance was adjusted at the end of each month after the pigs had been weighed. Samples of each feed were subjected to proximate chemical analysis (AOAC 2000).

Feed intake was recorded daily for each pen and pooled for a month (28 days). This was then used in the computation of

average daily feed intake. Similarly, weights taken at the end of every month were used in the computation of average daily weight gain. The average daily feed intake, divided by the average daily weight gain was calculated as the feed to gain ratio, that is, the weight of feed needed to produce one kilogram of gain. Similarly, the feed to gain ratio multiplied by the cost of a kilogram of feed was calculated as the cost of gain or the cost of feed needed to produce a kilogram of weight gain.

The effects of the various treatments on average daily weight gain, average daily feed intake, feed to gain ratio and cost of gain were investigated using analysis of variance (GENSTAT 1996), with the initial weight of pigs serving as a covariate.

Results

The nutrient analysis of reject cashew kernels, composition of experimental feeds, proximate analysis of experimental feeds are shown in Tables 1, 2 and 3, respectively. Means for the various treatments for average daily feed intake, average daily weight gain, feed to gain ratio and cost of gain are shown in Table 4. This is discusion. A growth rate of 0.32 – 0.40 kg day-1 is the norm on that diet. Analysis of variance revealed no significant differences ($P > 0.05$) between treatments with respect to average daily gain, average daily feed intake and feed conversion ratio. Cost of gain tended to decrease with the inclusion of reject cashew kernel in the diet, with treatment 0 RCK being significantly ($P < 0.05$) higher than the other two treatments and treatment 150 RCK also being significantly ($P < 0.05$) higher than treatment RCK 300.

Discussion

Compared with the analysis of Fetuga *et al.,* (1975) and Fanimo *et al.,* (2004), the reject cashew kernels used in this study had a higher level of fat, crude fibre and crude protein. This may have been due to the fact that the cashew nuts were from different sources. Reject cashew kernel is a 'full – fat' product with high energy and protein. As a result, as the level of reject cashew kernel increased in the diet, the amount of maize needed was reduced and at 300 gkg-1(300 RCK) there was no maize the diet. Maize from the diet is of major significance as it is an important staple food for humans in Africa. Animal feeds without maize, are therefore, desired.

Reject cashew kernel is a fairly new feed ingredient on the market but is bound to become more popular and available as the number of processing factories in Ghana increase. Being a 'full - fat' product, it supplies both energy and protein at appreciable levels in the diet and is a valuable feed ingredient. Because it is still relatively unknown, it is being sold at a relatively cheap price which caused a significantly decreased the feed price as its inclusion was increased. Although it was tested as far back as 1975, its use appears not to have caught on and there is a paucity of information on its use apart from the work of Fetuga *et al.* (1975) and Fanimo *et al.* (2004).

The performance of growing pigs fed 300 gkg-1 of reject cashew kernels in their diets indicates that it may be successfully fed at a higher level and there will be the need to investigate this. The nutrient analysis of reject cashew kernels indicates that it will probably be useful in poultry diets as well and this will need to be investigated. This study did not look at the effect on carcass quality and this will also need to be investigated.

Table 1: Nutrient content of cashew kernels

NUTRIENTS (g kg-1)	Fetuga et al., (1975)	Fanimo et al., (2004)	Current study
Dry matter	956	954.6	870
Crude protein	216	215	229
Ether extract	455	455	580
Crude fibre	23	23	27.5
Ash	38	38	20
Calcium	0.3	-	0.21
Phosphorus	8.4	-	7.3
Gross energy (MJ kg-1)	30.22	30.33	-
Estimated Metabolisable Energy (MJ kg-1)			5639

Table 2. Composition of experimental feeds

	Experimental feeds		
INGREDIENTS (g kg-1)	**0 RCK**	**150 RCK**	**300 RCK**
Reject cashew kernel	0	150	300
Maize	310	250	0
Wheatbran	300	241	245.2
Copra cake	150	100	100
Cocoa pod husk	40	150	300
Soyabean cake	80	50	0
Palm oil	50	0	0
Tuna meal	50	40	40
Oyster shell	13	13	10
Common salt	2.5	2.5	2.5
Vitamin/mineral mix	1.25	1.25	1.25
Synthetic lysine	2.25	1.25	0
Michochem	1	1	1
TOTAL	**1000**	**1000**	**1000**
CALCULATED ANALYSIS			
Digestible Energy (MJ Kg-1)	12.3	12.5	12.9
Crude protein (gkg-1)	173.9	170.2	171.8
Lysine (gkg-1)	10.0	10.2	10.6
Methione + cystine (gkg-1)	5.3	5.2	5.1
Calcium (gkg-1)	8.0	8.3	8.5
Available phosphorus (gkg-1)	6.6	6.6	7.4
Cost (GH¢ metric tonne-1)	430.00	360.00	280.00

NB

1. 1GH¢ = 0.69USD as atDecember 20, 2010.

2. Michochem is a commercial mould fixing agent which is added to feeds at a rate of 1 Kg per 1000 Kg of feed. It binds mycotoxins in vivo preventing them from causing harm to the animals.

Key
0 RCK - Control
150 RCK - 150 gkg-1 reject cashew kernel in feed
300 RCK - 300 gkg-1 reject cashew kernel in feed

Table 3: Proximate analysis of experimental feeds

	0RCK	150RCK	300RCK
Dry matter (g Kg^{-1})	880	880	875
g Kg^{-1}DM			
Organic matter	920	935	940
Crude protein	185	189	177
Ether extract	265	85	200
Crude fibre	63.2	119.6	163.3
NDF	525	600	610
ADF	440	550	365
ADL	180	225	250
Calcium	8.6	8.5	8.1
Phosphorus	7	6.2	6

Key
0 RCK - Control
150 RCK - 150 gkg-1 reject cashew kernel in feed
300 RCK - 300 gkg-1 reject cashew kernel in feed

Table 4: Feed intake and average daily weight gain

	0RCK	**150RCK**	**300RCK**	**Overall Mean**	**SED**	**SIG**
Average daily gain (Kg day-1)	0.39	0.39	0.38	0.39	0.012	NS
Average daily feed intake (Kg day-1)	2.31	2.22	2.15	2.23	0.066	NS
Feed conversion ratio	5.92	5.77	5.68	5.79	0.276	NS
Cost of gain (GH¢)	2.54a	2.08b	1.59c	2.07	0.098	*

Key
0 RCK - Control
150 RCK - 150 gkg-1 reject cashew kernel in feed
300 RCK - 300 gkg-1 reject cashew kernel in feed

Conclusion

In conclusion, reject cashew kernels appear to be a useful feed ingredient for growing pig diets and can be fed at levels of up to 300 gkg-1 of diet without any deleterious effects. It also has a sparing effect on the inclusion of maize in pig diets.

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References

Adomako D, Osei-Amaning E and Asante EG, 1999. Compound Animal Feeds based on Cocoa Pod Husk. *Tropical Science,* 39: 120-123.

Association of Official Analytical Chemists, 2000. Official Methods of Analysis. 17th edn. *Association of Official Analytical Chemists,* Gaithersburg, MD.

Attoh-Kotoku V, Donkoh A, Osafo ELK and Osei J, 2007. Varying levels of maize bran in diets on the performance and carcass characteristics of starter-grower pigs. In the Proceedings of the 2007 Conference of the *Ghana Society of Animal Production*, pp: 169 - 173.

Ayivi E, 2008. Use of selected agro-industrial by-products in the feeding system of pigs, Unpublished MSc. thesis, Kwame Nkrumah *University of Science and Technology,* Kumasi.

Barnes AR, Amega WK, Manu M and Rhule T, 1985. Utilization of cocoa husk meal by growing finishing pigs. In the Proceedings of the 1984 *International Cocoa Conference,* pp: 449 - 454.

Bruce J, Karbo N and Avornyo FK, 2004. Changes in pig production in Northern Ghana. In the Proceedings of the 2004 Ghana Animal Science Symposium, pp: 99 - 109.

Fetuga BL, Babatunde GM and Oyenuga VA, 1975. The feedingstuff potential of cashewnut scrap kernel meal. In the Proceedings of the 1974 Conference on *Animal Feeds of Tropical and Subtropical origin*, pp: 201-207.

Fanimo AO, Oduguwa OO, Adewunmi TE and Lawal AI, 2004. Utilization of diets containing cashew nut reject meal by weaner pigs. *Nigerian Journal of Animal Production,* 31(1): 22-26.

Lawes Agricultural Trust, 1996. Genstat 5 for Personal Computers, Version 3.2, 2nd edn. *Rothamsted Experimental Station,* United Kingdom.

Okai DB and Bonsi MLK, 1989. Sheanut cake as a substitute for maize in the diets of growing gilts. *Journal of the University of Science and Technology,* 9: 45 - 50.

Rhule SWA, 1995. Performance of weaner pigs on graded levels of palm kernel cake. *Legon Agricultural Research and Extension Journal*, 4: 105 - 108.

Rhule SWA, Okai DB, Addo Kwafo and Ameleke G, 2007. Feeding package for pigs in Ghana using AIBPs: Solution to feeding constraints.. In the Proceedings of the 2007 *Conference of the Ghana Society of Animal Production*, pp: 175-180.

COMPARATIVE STUDY OF SOME ZOOTECHNICAL CARACTERISTICS IN BAOULE, N'DAMA AND ZEBU CATTLE BREEDS IN THE CENTAL REGION OF CÔTE D'IVOIRE

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ETUDE COMPARATIVE DE QUELQUES CARACTERISTIQUES ZOOTECHNIQUES CHEZ LES BOVINS BAOULE, N'DAMA ET ZEBU DANS LA REGION CENTRE DE LA CÔTE D'IVOIRE

Resume

La présente étude a pour objectif de comparer les performances zootechniques des animaux de race Baoulé, N'Dama et Zébu. Des données portant sur les paramètres de production ont été collectées sur 105 *troupeaux* dont 40 troupeaux de bovins Baoulé, 31 de bovins N'Dama et 34 de bovins Zébu dans la région Centre. Les poids moyens (22,50 kg à la naissance, 184,32 kg au sevrage et 335,28 kg à la maturité) des bovins Zébu ont été significativement plus élevés que ceux des bovins N'Dama et Baoulé. Les bovins Baoulé ayant eu les poids moyens les plus faibles (12,18 kg à la naissance, 98,63 kg et 178,55kg à la maturité). En outre, les performances de croissance pondérale des bovins Zébu ont été significativement supérieures à celles des races taurines locales. Ces différences significatives pourraient constituer un atout majeur pour tout programme d'amélioration de la production bouchère impliquant en croisement raisonné entre le Zébu et ces races locales.

Mots clés : Performance, production, Baoulé, N'Dama, Zébu

Abstract

The *indigenous trypanotolerant* cattle (Baoulé and N'Dama) of Côte d'Ivoire are neglected by farmers who prefer Zebu cattle which are supposed to have a big size. Farmers estimate that Baoulé and N'Dama breeds are not productive due to their relative small size. The aim of this study aim was to compare zootechemical performances of Baoulé, N'Dama and Zebu cattle. Productive data was collected in 105 herds inclueding; 40 herds of Baoulé breed, 31 herds of N'Dama breed and 34 herds of Zebu cattle in the Central region. The avarage weights (22,50 kg at birth, 184,32 kg at weaning and 335,28 kg at mature age) of Zebu Cattle were significantly higher than the average weights of Baoulé and N'Dama breeds. The Baoulé breed presented the lowest average weights (12, 18 kg at birth, 98,63 kg at weaning and 178,55 kg at mature age). Moreover, the daily weight gains are significantly above those of indigenous taurine cattle. These significant differences could be a major advantage in any crossbreeding program including Zebu cattle and indigenous cattle.

Key words: Performance, productive, Baoulé, N'Dama, Zebu

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Introduction

La Côte d'Ivoire n'avait pas de tradition pastorale avant l'Indépendance. Cependant, un élevage national a été constitué en quelques décennies à partir de 1970. En effet, pour soutenir le développement de l'élevage dans ce pays, de nombreuses structures at infrastructures ont été mises en place. Il s'agit notamment de la Société de Développement des Productions Animales (SODEPRA), du Laboratoire National d'Appui au Développement Agricole (LANADA), l'IDESSA (Institut des Savanes) et le Centre National Ovin (CNO). Le cheptel ivoirien est constitué d'environ 1442000 bovins, 1487000 ovins, 1162000 caprins, 346000 porcins et 31 millions de volailles. Toutefois l'élevage reste encore une activité économique secondaire avec une contribution directe d'environ 4,5% au PIB agricole et 2% au PIB total. Il constitue néanmoins, une activité importante qui concourt à l'amélioration de la sécurité alimentaire, à la diversification et à l'augmentation des revenus des paysans et des éleveurs (MINAGRA, 2002).

Le cheptel bovin de la Côte d'Ivoire est majoritairement composé de deux races taurines locales (Baoulé et N'Dama) et de Zébus.

Le poids des zébus Peuhl adulte varie de 208 à 332 kg pour un âge compris entre 4 et 5 ans. Les zébus Maure pèsent en moyenne 305 kg. Le rendement carcasse est compris entre 53 et 57%. Ces animaux sont utilisés aussi bien pour la production de viande que pour la production laitière (Yapi-Gnaoré et al., 1996). L'aptitude dominante du bovin Baoulé est la production de viande. Son poids à l'âge adulte varie entre 189 et 283 kg avec un rendement carcasse pouvant atteindre 48 à 55% (Tidori et al., 1975). L'aptitude laitière est faible, avec une production moyenne de 3 litres de lait par jour. Le bovin N'Dama est un animal bien conformé pour la boucherie Le poids moyen à la naissance est de 17 kg et le poids adulte, généralement de 255 kg, peut atteindre 300 kg avec un rendement carcasse variant entre 48% et 55% (Yapi-Gnaoré et al., 1996). La vache N'Dama est une mauvaise laitière avec une production moyenne de 4 litres de lait par jour. La particularité biologique de ces races locales est leur résistance à la trypanosomose animale africaine. Malheureusement, elles sont menacées d'absorption par les Zébus, par la faute des éleveurs qui les soumettent à des croisements intenses. Ces opérations de métissage font craindre la baisse de la trypanotolérance. La perte d'une telle spécificité biologique dans une zone d'élevage fortement infestée par les glossines provoque une baisse sensible de la productivité des élevages, surtout au niveau des paysans qui possèdent de petites unités (Hoste, 1992). D'où la nécessité de sensibiliser les producteurs. Cela passe par l'approfondissement de la connaissance des caractéristiques des races bovines locales. Cependant, les caractéristiques zootechniques et la productivité de ces races sont peu connues. En effet, les publications disponibles ne sont pas récentes. Par conséquent, la présente étude a pour objectif de déterminer les performances de production des races bovines, en milieu paysan afin de contribuer à la connaissance des bovins locaux.

Materiel et Methodes

Zone d'étude

Les travaux ont été réalisés dans des fermes villageoises situées dans la périphérie des villes de Bouaké et Katiola où la densité de fermes et de populations bovines est élevée. Le choix des fermes a été fait sur la base de la disponibilité des éleveurs, la présence dans le troupeau d'au moins 20 femelles reproductrices et de l'existence dans les fermes d'un minimum de commodités c'est-à-dire parc de nuit, couloir de contention, abreuvoir.

Les localités de Bouaké et Katiola sont situées dans la région Centre de la Côte d'Ivoire. Cette région est caractérisée par

des savanes arborées ou arbustives et des forêts galléries le long des cours d'eau. Elle constitue une zone de transition entre la forêt et la savane (zone soudano-guinéenne). Les précipitations qui varient de 1 000 à 2 500 mm, sont réparties en deux saisons de pluie d'avril à juin et d'août à octobre (Yapi-Gnaoré et al., 1996). La végétation est constituée en majorité de Lephira lanceolata, Isoberlinia doka, Daniellia oliveri, Parinari curatellifolia, Uapaca togoensis, Detarium microcarpum et de jachères anciennes à Andropogon gayanus (César, 1981).

Matériel animal

L'étude a porté sur des animaux mâles et femelles de races taurines Baoulé, N'Dama et Zébu dont les performances de production ont été contrôlées en milieu paysan. Les données ont été collectées dans 40 troupeaux de bovins Baoulé, 31 troupeaux de N'Dama et 34 troupeaux de Zébu, au cours de la période allant de mars 1999 à septembre 2002. Les animaux de race N'Dama présente en général une robe fauve ou fauve-pie. Ils ont une taille moyenne dont la hauteur au garrot varie de 108 à 117 cm pour des animaux d'âge compris entre 4 et 8 ans. Le périmètre thoracique varie de 140 à 170 cm (Sokouri et al., 2007). Le bovin Baoulé présente une robe de couleur variable; blanche, noire, pie-noire, noire-pie, pie rouge, rouge-pie et quelques fois rouge noire. La race Baoulé est également caractérisée par son petit format ; la hauteur au garrot a varie de 92 à 113 cm et le périmètre thoracique de 129 à 161 cm pour des animaux de plus de 4 ans (Sokouri et al., 2007). La couleur de la robe du bovin Zébu est très variable. C'est un animal haut sur pattes ; la hauteur au garrot varie de 118 à 130 cm pour des animaux de plus de 4 ans. Le périmètre thoracique varie de 143 à 172 cm (Sokouri et al., 2007).

Conduite et gestion des animaux

Chaque jour, les animaux ont été conduits sur pâturage naturel de huit (08) heures à 16 heures. En saison sèche, leur régime alimentaire du pâturage a été complété avec des résidus de récoltes issus des champs de cultures. Les veaux non sevrés ont été conduits séparément de neuf (09) heures à 13 heures. Après sevrage, la période de pâturage a été prolongée jusqu'à 16 heures. A l'âge de 12 mois, les taurillons ont été séparés des génisses. Pour les fermes dans lesquelles il existe des troupeaux d'animaux de races différentes, ces troupeaux ont été conduits séparément afin d'éviter tout contact entre taureaux et vaches de races différentes. Les animaux ont été déparasités et vaccinés régulièrement. Les animaux ont été déparasités une fois par mois en saison sèche et deux fois par mois en saison des pluies contre les tiques.

Caractères mesurés

Les caractères de production étudiés ont été :

- le poids à la naissance, au sevrage (12 mois), à maturité (36 mois) ;
- le gain moyen quotidien (GMQ) des six premiers mois (GMQ06), entre le sixième mois et le 12ième mois (GMQ12), entre le sevrage et le 24ième mois (GMQ24), et entre 24 mois et l'âge de la maturité (GMQ36) ;
- le rendement carcasse.

Les performances de chaque animal ont été enregistrées sur une fiche de suivi comportant les renseignements suivants :

- numéro de l'animal ;
- numéro de la mère;
- mois de naissance;
- année de naissance;
- sexe;
- poids à la naissance;
- poids mensuel.

Tous les veaux ont été pesés à la naissance et à la fin de chaque mois jusqu'à l'âge de 24 mois, puis trimestriellement entre 24 mois et 48 mois. Le poids à 36 mois a été

considéré comme le poids à l'âge adulte (maturité) des animaux.

Le poids a été mesuré à l'aide d'une balance, ou estimé à l'aide d'un ruban barymétrique. La barymétrie qui est une mesure approximative, est la détermination du poids vif des animaux par des mensurations avec un mètre ruban. Cependant, elle est d'une grande utilité puisque la bascule servant à la pesée des bovins est un instrument très lourd difficilement transportable sur le terrain. Le ruban barymétrique a été donc mis au point sur la base de la corrélation entre le périmètre thoracique et le poids. Par conséquent, ce ruban donne directement le poids de l'animal en kg par la mesure de son périmètre thoracique (IEMVT, 1971).

Le rendement carcasse est le pourcentage exprimant le rapport entre le poids de la carcasse c'est-à-dire la tête, les pieds et viscères exclus d'un animal et le poids vif de cet animal. Le rendement carcasse a été déterminé à partir de la formule suivante :

$$\text{Rendement} = \frac{\text{Poids carcasse (kg)}}{\text{Poids vif après 12 heures de jeun (kg)}} \times 100$$

Dans chaque troupeau, un animal (mâle ou femelle) a été tiré de façon aléatoire, puis il a été abattu afin de calculer son rendement carcasse.

Traitement statistique des données

Les données ont été analysées à l'aide du logiciel SPSS 10.0.

Yijkl = Ri + Sj + Tk + Sel + eijklm, où

Yijkl = Caractères mesurés ;

Ri = race ; i = 1 à 3 ;

Sj = saison ; j = 1 à 2 ;

Tk = troupeau ; k = 1 à 25 ;

Sel = sexe ; l = 1 à 2 ;

L'analyse de la variance a été obtenue par la procédure du modèle linéaire général (GLM). Les moyennes des différents paramètres de production ont été calculées et comparées deux à deux par le test de t.

Resultats

Influence des facteurs de variation sur les caractères mesurés

L'analyse par le modèle linéaire général a révélé que la race et le troupeau ont un effet hautement significatif (P<0,01) sur le poids à âge type et les différents gains moyens quotidiens. L'effet de ces deux facteurs de variation sur le rendement carcasse a été significatif (P<0,05). Si l'effet de la saison a été significatif (P<0,05) sur le poids à la maturité, le GMQ36 et le rendement carcasse, il a été hautement significatif sur les autres caractères de production. Le sexe a eu un effet hautement significatif (P<0,01) sur le poids à la maturité, les GMQ24 et GMQ36. Cependant, l'effet du sexe n'a pas été significatif (P>0,05) sur tous les autres caractères étudiés (Tableau 1).

Performances de production bouchère

L'analyse de variance a indiqué une différence hautement significative entre les trois races aussi bien pour le poids à la naissance, le poids au sevrage que pour le poids à l'âge adulte. Le poids à la naissance des bovins Zébu qui a été en moyenne de 22,50 kg a été le plus élevé (P<0,01). Le poids à la naissance des veaux Baoulé avec une moyenne de 12,18 kg a été le plus faible. Chez la race N'Dama, le poids à la naissance a variant de 12 à 23 kg avec une moyenne de 16,95 kg (Tableau 2). Au sevrage, le poids des bovins Zébu a été également significativement le plus élevé (P<0,01) avec une moyenne de 184,32 kg, tandis que le poids des bovins Baoulé qui a été de 98,63 kg en moyenne a été le plus faible (Tableau 2). A l'âge de la maturité correspondant à 36 mois, le poids des bovins Baoulé a été en moyenne de 178,55 kg, il a varié entre 114 et 265 kg. Le poids moyen adulte des bovins N'Dama a été de 294,30 kg avec une variation de 244 à 351 kg. Chez les bovins Zébu, le poids moyen adulte a été de 335,28 kg, variant entre 265 et 391 kg. Les bovins Baoulé sont des animaux de petit

Tableau 1: Significativité de l'effet des facteurs de variation sur les caractères de production

Source de Variation	Poids (kg)			Gain Moyen Quotidien (grammes/jour)				Rendement carcasse (%)
	P0	P12	P36	GMQ06	GMQ12	GMQ24	GMQ36	
Race	**	**	**	**	**	**	**	*
Troupeau	**	**	**	**	**	**	**	*
Saison	**	**	*	**	**	**	*	*
Sexe	NS	NS	**	NS	NS	**	**	NS

P0 : Poids à la naissance ; P12 : Poids au sevrage ; P36 : Poids à la maturité (ou à l'âge adulte)
GMQ06 : gain moyen quotidien des six premiers mois
GMQ12 : gain moyen quotidien entre le sixième mois et l'âge au sevrage (12 mois d'âge)
GMQ24 : gain moyen quotidien entre l'âge au sevrage et le 24ième mois d'âge
GMQ36 : gain moyen quotidien entre le 24ième mois et l'âge à la maturité (36 mois d'âge)
NS : Effet non significatif au seuil de 5 %
* : Effet significatif au seuil de 5 %
** : Effet significatif au seuil de 1 %

Tableau 2 : Performances bouchères des races locales (Baoulé et N'Dama) et de la race Zébu.

Race	Poids à la naissance (kg)			Poids au sevrage (kg)			Poids à l'âge adulte (kg)		
	Effectif	Moy-enne	Ecart-type	Effectif	Moy-enne	Ecart-type	Effectif	Moy-enne	Ecart-type
Baoulé	77	12,18 c	0,25	76	98,63c	3,33	73	178,55 c	8,05
N'Dama	69	16,95 b	0,44	69	130,41b	2,47	66	294,30 b	5,61
Zébu	58	22,50 a	0,42	54	184,32a	2,42	51	335,28 a	5,75

Les moyennes de la même colonne portant des lettres différentes diffèrent significativement.

format comme l'indique le poids moyen à la maturité. Le poids moyen de ces animaux a été significativement plus faible que ceux des animaux N'Dama et Zébu. Les bovins Zébu sont des animaux de grand format dont le poids moyen à la maturité a été significativement plus élevé (P< que celui des bovins N'Dama (Tableau 2).

Le rendement carcasse a varié de 48 à 60 %, avec une moyenne de 52,05 % chez le bovin Baoulé. Il a été en moyenne de 53,55 %, avec une variation allant de 50 à 60 % chez le bovin N'Dama alors que chez les Zébu, ce rendement a été de 54,80 % en moyenne, variant entre 50 et 62%. Les résultats ont montré qu'entre les trois races, la race Baoulé a le rendement carcasse le plus faible. Cependant, le rendement carcasse des bovins N'Dama n'a pas été significativement différent de celui des animaux Zébu.

Croissance pondérale

Les résultats obtenus ont indiqué que de la naissance au douzième mois d'âge, les animaux Baoulé et N'Dama ont la même évolution pondérale. Les animaux Zébu ont connu une croissance légèrement plus rapide que les animaux Baoulé et N'Dama entre la naissance et le sixième mois. Du sixième au douzième mois, la différence de croissance a été plus nette entre les bovins Zébu et Baoulé, d'une part et entre les bovins Zébu et N'Dama, d'autre part. A partir du douzième mois, la croissance pondérale des animaux N'Dama a été supérieure à celle des bovins Baoulé et elle n'a pas été significativement différente de celle des Zébu. Par ailleurs, la croissance

Tableau 3 : Classement des races bovines en fonction des paramètres de croissance

Race	GMQ06 (g/j)	GMQ12 (g/j)	GMQ24 (g/j)	GMQ36 (g/j)
Baoulé	274c	154,45c	193c	139c
N'Dama	389,5b	187b	290,33b	233,67a
Zébu	460,54a	266a	328,5a	202,23b

GMQ06 : gain moyen quotidien des six premiers mois
GMQ12 : gain moyen quotidien entre le sixième mois et l'âge au sevrage (12 mois d'âge)
GMQ24 : gain moyen quotidien entre l'âge au sevrage et le 24ième mois d'âge
GMQ36 : gain moyen quotidien entre le 24ième mois et l'âge à la maturité (36 mois d'âge)

pondérale des animaux N'Dama a été plus rapide que celle des bovins Zébu entre 24ème et le 36ème mois. En effet, l'analyse du gain moyen quotidien (GMQ) a montré que la croissance a été significativement plus lente (P<0,01) chez les bovins Baoulé. Ces animaux ont le GMQ le plus faible à tous les intervalles d'âges. Par contre, l'évolution pondérale des bovins Zébu a été la plus rapide. Les GMQ de ces animaux ont été significativement (P<0,01) les plus élevés les six premiers mois (460,54 g/j), entre 6 et 12 mois (266 g/j), entre 12 et 24 mois (328,5 g/j) et enfin entre 24 et 36 mois (328 g/j). Cependant, entre 24 et 36 mois d'âge, le GMQ des bovins N'Dama qui a été de 233,67 g/j en moyenne a été significativement plus élevé que celui du bovin Zébu dont le GMQ36 a été 202,23 (Tableau 3). En outre, la tendance de l'évolution du gain de poids corporel a été similaire chez les trois races. En effet, les GMQ des six premiers mois ont été plus élevés que ceux obtenus entre le sixième mois et le 12ième mois. Par ailleurs, au niveau de chacune des races étudiées, la croissance des animaux entre le sevrage et le 24ième mois a été plus rapide que celle observée entre le 24ième mois et l'âge à la maturité (Tableau 3).

Discussion

Les résultats de la présente étude ont indiqué que les caractères de production mesurés ont significativement varié en fonction de la saison. Les poids à différents âges et les gains de poids corporels ont été plus élevés en saison de pluie qu'en saison sèche. Ceci est peut être imputable à la longueur des différentes saisons sèches au cours de la période d'étude. L'allongement de la durée de la saison sèche dû au changement climatique diminue encore davantage l'apport en aliment de base suite au desséchement des pâturages naturels et a leur lignification (Hatun *et al.,* 2007). En outre, selon (Pamela *et al.*, 2001), les facteurs environnementaux qui influencent la capacité de production sont connus. Il s'agit de problèmes pathologiques, de variations climatiques, etc. C'est ainsi que les performances varient en fonction du milieu d'élevage. En plus des principaux facteurs environnementaux énumérés par ces auteurs, il serait judicieux d'ajouter la gestion du troupeau. En effet, la présente étude a montré que l'effet du troupeau a été significatif sur tous les caractères de production. Ces résultats sont corroborés par Chikhi et Boujenane (2003) qui ont réalisé des travaux sur les performances zootechniques de races ovines. Ces auteurs ont montré que le mode de conduite des animaux et les disponibilités alimentaires expliquent en grande partie les différences de poids et de GMQ. Aussi, l'hétérogénéité des performances entre exploitations peut être en partie reliée au fait qu'au-delà du matériel animal et des techniques, c'est aussi le métier d'éleveur qui est en cours de construction (Choisis *et al.,* 2008). En outre, les différences significatives des performances en fonction de la race laissent suggérer que quoi qu'il en soit, les performances zootechniques témoignent du potentiel génétique des races. Le facteur

"race" est donc déterminant dans la performance zootechnique (Somda, 2001). En effet, les bovins Zébu qui n'étaient pas dans leur zone naturelle d'élevage ont présenté les meilleures performances pondérales et de croissance. Les poids moyens à âges types et les GMQ de ces animaux ont été significativement plus élevés que ceux des races locales Baoulé et N'Dama. Le poids moyen à la naissance des bovins Zébu qui a été de 22,50 kg est comparable à celui de 23 kg rapporté par Yapi-Gnaoré *et al.* (1996). Le poids à l'âge adulte obtenu par ces auteurs varie de 208 à 332 kg pour des animaux d'âge compris entre quatre et cinq ans, alors que les valeurs obtenues dans cette étude ont été de 265 à 391 kg. Cette différence pourrait être due à l'amélioration de l'environnement d'élevage. Dans les deux cas, les données ont été collectées en milieu réel. Cependant, dans le cas de la présente étude, les élevages ont bénéficié d'un encadrement ou d'un suivi sanitaire durant tout le déroulement des travaux et une complémentation alimentaire a été régulièrement apportée aux animaux.

Le poids à la maturité des bovins Baoulé qui a varié de 114 à 265 kg avec une moyenne de 178,55 kg est inférieur à celui de 189 et 283 kg rapporté par Yapi-Gnaoré et al. (1996). Le rendement carcasse est comparable à celui obtenu au CRZ (Centre de Recherches Zootechniques) de Minankro-Bouaké, variant entre 48 et 55 % (Tidori *et al.*, 1975).

Les valeurs des paramètres de production obtenues chez la race N'Dama sont comparables à celles rapportées par (Yapi-Gnaoré et al., 1996). D'après ces auteurs, le poids moyen à la naissance est de 17 kg et le poids adulte, généralement de 255 kg, peut atteindre 300 kg. Le rendement carcasse variant entre 48% et 55%. Youssao et al. (2000) qui ont mesuré les performances zootechniques de bovins N'Dama introduits à la ferme de l'Okpara (Bénin), ont trouvé un poids moyen à la naissance de 17.4 kg.

Toutes les performances pondérales et de croissance observées dans la présente étude ont confirmé le fait que les animaux de race Baoulé et N'Dama ont un format moins important que celui des animaux Zébu. Cependant, ces races locales ont présenté de bons potentiels génétiques qui peuvent être valorisés, même à travers des programmes d'amélioration génétique destinés à des systèmes de production à faibles intrants comme l'ont démontré (Bosso et al., 2007) pour la race N'Dama en Gambie.

Conclusion

Toutes les performances pondérales et de croissance obtenues dans la présente étude ont confirmé la supériorité significative des bovins Zébu sur les taurines locales (Baoulé et N'Dama) qui ont une conformation moins importante que celle des animaux Zébu. Cependant, les animaux Baoulé et N'Dama ont eu une bonne évolution pondérale dont la tendance est quasiment similaire à celle des Zébu. En outre, les résultats obtenus ont montré que malgré les différences significatives des poids vifs des races Baoulé, N'Dama et Zébu, les rendements carcasse de ces races sont plutôt similaires montrant qu'il n'y a pas de différence significative entre les bovins N'Dama et Zébu.

Par ailleurs, ces différences significatives pourraient constituer un atout majeur pour tout programme d'amélioration de la production bouchère impliquant en croisement raisonné les Zébu et les races taurines locales. Car une quasi dominance ou encore une hyper dominance au niveau des performances pondérales et de croissance est évidente (Scholtz et Theunissen, 2010).

References Bibliographiques

Bosso NA, Corr N, Njie M, Fall A, van der Waaij, van Arendonk JAM, Jainter J, Dempfle L et Agyemang K, 2007. The N'Dama cattle genetic improvement programme: a review. *Animal Genetic Resources Information* (AGRI), (40) : 65-69.

César J, 1981. Cycles de la biomasse et des repousses après coupe en savane de Côte d'Ivoire. Rev. Elev. *Méd. Vét. Pays Trop*, 34 (2) : 187-198.

Chikhi A et Boujenane I, 2003. Performances de reproduction et de production des ovins de race Boujaâd au Maroc. Revue Elev. *Méd. Vét. Pays trop.,* 56 (1-2) : 83-88.

Choisis JP, Lasalle C, Messad S et Grimaud P, 2008. Performances zootechniques et pratiques des éleveurs en élevage bovin naisseur à la Réunion. Revue Elev. *Méd. Vét. Pays trop*., 61 (2) : 89-96.

IEMVT, 1971. Manuel Vétérinaire des agents techniques de l'élevage tropical. Institut d'Elevage et de Médecine Vétérinaire des pays tropicaux (IEMVT) (1 st éd.). Ministère de la coopération. République française, 519 p.

Hatun GG, Hornick JL et Detilleux J, 2007. Aspects zootechniques de l'élevage bovin laitier au Burundi : présent et futur. *Ann. Med. Vét.,* 151. 150.

Hoste CH, 1992. Contribution du bétail trypanotolérant au développement des zones affectées par la trypanosomiase animale africaine. *FAO World Anim. Rev.,* 70 : 34.

MINAGRA, 2002. Rapport National sur l'État des Ressources Zoogénétiques. Ministère de l'Agriculture et des Ressources Animales (MINAGRA). *République de Côte D'Ivoire,* 77p.

Pamela KB, Njwe RM, Bayemi PH, Ndi C, Imelé H et Kameni A, 2001. Effets de la saison de vêlage et de quelques paramètres zootechniques sur la production laitière dans les hauts plateaux de l'Ouest du Cameroun. *Revue Elev. Méd. Vét. Pays trop.,* 54 (1) : 55-61.

Scholtz M.M. et Theunissen A, 2010. The use of indigenous cattle in terminal cross-breeding to improve beef cattle production in sub-saharan. *Animal Genetic Resources Information* (AGRI), (46) : 33-39.

Sokouri DP, Loukou NE, Yapi-Gnaoré CV, Mondeil J et Gnangbé F, 2007. Caractérisation phénotypique des bovins à viande (Bos taurus et Bos indicus) au centre (Bouaké) et au nord (Korhogo) de la Côte d'Ivoire. *Animal Genetic Resources Information* (AGRI), (40) : 43-53.

Somda J, 2001. Performances zootechniques et rentabilité financière des ovins en embouche au Burkina Faso. *Biotechnol. Agron. Soc. Environ,* 5 (2) : 73–78

Tidori E., Serres H., Richard D. et Ajuziogu J. 1975. Etude d'une population taurine de race Baoulé en Côte d'Ivoire. *Rev Elev Méd Pays Trop,* 28 (4) : 499.

Yapi-Gnaoré CV, Oya BA et Ouattara Z, 1996. Revue de la situation des races d'animaux domestiques de Côte d'Ivoire. *Animal Genetic Resources Information* (AGRI), (19): 99-118.

Youssao AKI, Ahissou A et Touré Z, 2000. Introduction de la race bovine N'Dama à la Ferme Elevage de l'Okpara au Bénin. Quelques performances *zootechniques*. *Animal Genetic Resources Information* (AGRI), (27) : 17-25.

EFFECT OF AQUEOUS EXTRACT OF SPONDIAS MOMBIN ON THE SPERMIOGRAM OF WISTAR RATS

EFFET DE L'EXTRAIT AQUEUX DE SPONDIAS MOMBIN SUR LE SPERMOGRAMME DE RATS WISTAR

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Abstract

Twenty-five male rats were divided into five groups (A-E) and treated for twenty-one days with aqueous leaf extract of *Spondias mombin* which is a fruit tree whose leaf extract is commonly consumed as local remedies in traditional medical practice. Graded dosages of the extracts were assigned to the groups as follows: 200 mg/ kg, 400 mg/ kg, 600 mg/ kg and 800 mg/ kg for groups B,C,D and E, respectively. Group A rats which served as control were given distilled water. Thereafter, *spermiogram* (mass activity, progressive motility, live-dead and morphology) was evaluated. Rats treated with aqueous extract of *Spondias mombin* at 600 mg/ Kg body weight had the highest ($p<0.05$) *spermatozoa* concentration of 40.51×106 cells/ ml. Compared with other groups A, B, C, and E which had 21.48 ×106, 29.58 ×106, 33.56 ×106, 35.81×106 cells / ml, respectively.
At dosage of 800 mg/Kg highest ($p<0.05$) sperm motility of 70 ± 4.81% was observed. Compared with other groups A,B C and D which had 65.0 ± 0.00, 65.0 ± 0.00, 67.5 ± 6.71 and 68.0 ± 2.89%, respectively. The percentage average livability of the control group and of those given 200 mg / kg BW, 70 ± 23.45 and 70 ± 23.09, respectively, were lower than those for groups C (77.5 ± 15.55), D (87.5 ± 17.68) and E (80.0 ± 17.32) with group D having the highest average percentage livability. Coiled tail was the predominant abnormality in groups A, B and E constituting 40.3%, 24.3% and 30.0% of the total abnormalities, respectively. The work recommended *aqueous* extract of *Spondias mombin* at concentration of 600mg/ml for of fertility enhancement in male animals.

Key words: Effect, *Aqueous*, Extract, *Spondias mombin, Spermiogram, Wistar* rats

Résumé

Vingt-cinq rats mâles ont été divisés en cinq groupes (de A à E) et traités pendant vingt et un jours avec une solution aqueuse de feuilles de Spondias mombin, un arbre fruitier dont le jus extrait des feuilles est couramment consommé comme remède dans la médecine traditionnelle. Les dosages proportionnels desdits extraits de Spondias Mombin ont été administrés aux différents groupes en fonction des critères ci-après : 200 mg par kg, 400 mg par kg, 600 mg par kg et 800 mg par kg pour les groupes B, C, D et E respectivement. Les rats du groupe A appelés à servir de témoins n'ont reçu que de l'eau distillée. L'équipe de recherche a procédé, par la suite, à l'évaluation du spermogramme (analyse de l'activité en masse, de la motilité progressive, des substances vivantes et mortes et de la morphologie des spermatozoïdes). Les rats auxquels a été administrée la solution aqueuse de Spondias mombin à 600 mg / kg de poids corporel présentaient la concentration la plus

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élevée (p <0,05) de spermatozoïdes de 40,51 × 106 cellules par ml par rapport à d'autres groupes A, B, C et E qui présentaient 21,48 × 106, 29,58 × 106, 33,56 × 106, 35,81 × 106 cellules par ml, respectivement. La motilité la plus élevée (p <0,05) des spermatozoïdes de 70 ± 4,81%, a été relevée lors du dosage de 800 mg de solution de Spondias mombin par kg, par rapport à d'autres groupes A, C et D qui présentaient des motilités de spermatozoïdes de l'ordre de 65,0 ± 0,00, 65,0 ± 0,00, 67,5 ± 6,71 et 68,0 ± 2,89% respectivement. Le pourcentage moyen d'espérance de vie du groupe de rats témoins et celui de ceux ayant reçu 200 mg de Spondias mombin par kg de poids corporel, 70 ± 23,45 ± 23,09 et 70 respectivement, ont été inférieurs à ceux des groupes C (77,5 ± 15,55), D (87,5 ± 17,68) et E (80,0 ± 17,32). L'on constate par ailleurs que les rats du groupe D présentent le pourcentage moyen le plus élevé d'espérance de vie. L'anomalie prédominante relevée dans les groupes A, B, et E respectivement constituées de 40,3%, 24,3% et 30% de l'ensemble des anomalies est la queue enroulée. Les travaux de recherche ont recommandé l'administration de la solution aqueuse de Spondias mombin à une concentration de 600mg par ml comme source d'amélioration de la fertilité chez les mâles.

Mots clés: Effet, solution aqueuse, extrait aqueux, Spondias mombin, spermogramme, rats Wistar

Introduction

Spondia mombin is a fructiferous tree that belongs to the family *Anacardiacae*. It grows in the coastal areas and in the rain forest into a big tree of up to 15–22mm in height. It is common in Nigeria, Brazil and several other tropical forests of the world with high genetic variability among populations (Ayoka *et al., 2008)* Phytochemical screening of the plant's leaf extracts using standard methods (Sofowora, 1982) shows presence compositions of saponins, tannins, alkaloids, flavonoids, phytate and cyanogenic glycosides been attributed to compounds (Ayoka *et al.*, 2008). The alkaloids affect glucagon, thyroid stimulating hormone and inhibit certain mammalian enzymatic activities (Okaka *et al*., 1992). Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity. These have also been associated with oxytocic and abortifacient activity of the plant's leaf extract (Offiah and Anyanwu 1989).

Reports have revealed the anxiolytic effect (Ayoka *et al*., 2005), sedative, antiepileptic and antipsychotic effects of the leave extract of *Spondia mombin* in mice and rats (Ayoka *et al.,* 2006). Raji *et al.* (2006) in their study showed the antifertility action of aqueous *Spondia mombin* bark extract. They reported a marked dose dependent reduction in epididymal sperm progressive motility, sperm count, viability (alive/dead ratio) and a dose-dependent increase in percentage abnormal spermatozoa. Epididymal α- glucosidase activity was significantly reduced. However, cessation of treatment resulted in full recovery within four weeks. The indiscriminate use of this herbal preparation among the locals is so rampant hence the need to study the likely effect it may have on the reproductive viability of the male. The male animal model (Wistar rat) used reaches puberty at 40 to 60 days of age. Descent of the testes usually occurs between 30 and 60 days. Sperm counts vary by strain (Wilkinson *et al.,* 2000). The male rat has an os penis and the following accessory sexual organs: ampulla, seminal vesicles, prostate, bulbo-urethral glands, coagulating glands, and preputial glands. The coagulating gland and prostatic and vesicular secretions are responsible for the copulation plug, a firm plug deposited in the vagina of the female after copulation. (This plug, when found outside the female rat, is capsule-shaped and approximately 5 mm long.). The work sought to determine the effect of repeated dosing of the leaf extract of *Spondias mombin* would have on the male reproductive parameters.

Materials and Methods

Plant collection and preparation of aqueous extract

Fresh leaves of *Spondias mombin* were collected at the University of Agriculture, Abeokuta, Ogun State of Nigeria in the month of June. The leaves were rinsed in clean water and air-dried (at room temperature until they crumbled to touch) to a constant weight. The dried leaves were ground into a fine powder using a mechanical grinder, packaged in glass jars and stored at 4°C as described by.

Aqueous extract was produced using water as extraction medium. The plant was soaked in water for three days. It was filtered using sieve to separate the leave particles. The filtrate was diluted again and filtered. The filtrate was pre-concentrated by heating to evaporate excess water until it turned into paste. The paste was then collected and stored at 4°C, refrigerator.

Animals

Male Wistar rats weighing 180 - 250 g were used in the study. All the experimental procedures were done following the experimental guidelines of Institutional Animal Ethics Committee (IAEC). Twenty-five male rats were used. The rats were housed in standard cages and fed *ad-libitum* with commercially prepared feed. Water was also given ad- libitum.

Two weeks of stabilization was observed. The male rats were divided into five groups (A to E) and treated for 21 days. Graded dosages of the extracts were assigned to the groups as follows: 200 mg/ kg, 400 mg/ kg, 600 mg/ kg and 800 mg/ kg for groups B,C,D and E respectively. Group A rats which served as control were given distilled water. Thereafter, spermiogram (mass activity, progressive motility, live-dead and morphology) was evaluated.

Spermiogram

The rats were anaesthetized with ether before sacrificing through cervical dislocation. In performing orchidectomy, a pre-scrotal incision was made and the testicles were milked out of the incision site to expose them. Semen samples were thereafter collected from the caudal epididymis using a method similar to that described by. The samples were analyzed immediately after collection.

A drop of buffered semen was placed on a clean glass slide before applying cover slip. It was viewed at X40 objective for gross motility of spermatozoa. Nigrosin- Eosin stained smear was also prepared by placing a drop of the epididymal spermatozoa on a clean glass slide and stained with Nigrosin-Eosin to observe the live- dead ratio. In like manner, Wells and Awa stain stained smear was prepared to examine the morphology of the spermatozoa.

Statistical Analysis

Student t-test was used to analyze the data (Steel and Torrie,1996). The difference of the means were considered significant at $p<0.05$. SPSS 16.0 (©2007 SPSS

Table 1: Effect of *aqueous* extract of *S. mombin* on the concentration of *spermatozoa* of groups A, B, C, D and E (± SD)

Group	Dosage (mg/Kg)	Average Concentration 1	Average Concentration 2
A	Distilled water	21.48 ± 8.26	18.6
B	200	31.0 ± 17.01	29.58
C	400	35.0 ± 21.87	33.56
D	600	41.4 ± 24.39	40.51
E	800	39.8 ± 25.40	35.81
Mean ±SD		31.61± 8.28	

1 = (×106 sperm cells/ml)

2 = adjusted by Testicular weight (×106 sperm cells/gml)

Table 2: Effects of *Aqueous* Extract of *S. mombin* on the average Motility, Concentration and Livability of *spermatozoa* of Rats in Groups A (control), B, C, D and E (± SD).

Group / Dosage (mg/ Kg)	Average Motility (%)	Average Concentration ($\times 10^6$ Cells/ml)	Average Liveability (%)
A (Distilled water)	65.0 ± 0.00	18.6 ± 8.26	70.0 ± 23.45
B (200 mg/Kg)	65.0 ± 0.00	29.58 ± 17.01	70.0 ± 23.09
C (400 mg/Kg)	67.5 ± 6.71	33.56 ± 21.87	77.5 ± 15.55
D (600 mg/Kg)	68.0 ± 2.89	40.51 ± 24.39	87.5 ± 17.68
E (800 mg/Kg)	70.0 ± 4.81	35.81 ± 25.40	80.0 ± 17.32

Table 3 : One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
conc	5	31.6120	8.27698	3.70158
mot	5	67.1000	2.13307	.95394
live	5	77.0000	7.37394	3.29773

Table 4 : Mean Difference

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
conc	8.540	4	.001	31.61200	21.3348	41.8892
mot	70.340	4	.000	67.10000	64.4514	69.7486
live	23.349	4	.000	77.00000	67.8440	86.1560

incs licensed to Team EQX 6th birthday 1337) was used.

Results

It was observed that rats treated with aqueous extract of *S. mombin* at 600 mg/Kg body weight had the highest spermatozoa concentration of 40.51×10^6 cells/ ml. This was highly significant ($p<0.05$) compared with other groups A, B, C, and E which had 21.48 $\times10^6$, 29.58 $\times10^6$, 33.56 $\times10^6$, 35.81$\times10^6$ cells / ml, respectively (Table 1, 3 and 4).

It was also observed that rats given the highest dosage 800 mg/Kg had highest sperm motility of 70 ± 4.81%which was significant ($p<0.05$) compared to other groups A,B C and D which had 65.0 ± 0.00, 65.0 ± 0.00, 67.5 ± 6.71 and 68.0 ± 2.89%, respectively (Table 2, 3 and 4).

At $p<0.05$, significantly the percentage average liveability of the control group and those given 200 mg / Kg (body weight) 70 ± 23.45 and 70 ± 23.09 were lower than those for groups C (77.5 ± 15.55), D (87.5 ± 17.68) and E (80.0 ± 17.32) with group D having the highest average percentage liveability (Table 2, 3 and 4).

Coiled tail was the predominant abnormality in groups A, B and E constituting 40.3%, 24.3% and 30.0% of the total abnormalities, respectively.

Discussion and Conclusion

The study showed that the percentage motility of the control experiment (group A), 65 ± 0.00 %, was lower than that reported by Oyeyemi *et al* (2006) for rats. The concentration 21.48

$\times 10^6$ cells / ml and percentage liveability 70.0 ± 23.44 % of the control group was lower than 67.40 $\times 10^6$ and 76.0 ± 2.40 % reported for concentration and percentage liveablility, respectively, of rats by (Oyeyemi *et al.*, 2006).

The study also showed that group D given 600 mg /Kg of *aqueous S. mombin* extract had a higher percentage liveability of 87.5 ± 17.68 % than the control group 65.0 ± 0.00 % but lower than 88.0 ± 3.39 % reported by (Oyeyemi *et al.,* 2006). The studys report is at variance with (Raji *et al.,* 2006) submission (using bark extract) that the plant reduces the reproductive potential of the Wistar rat. Coiled tail was predominant in the control group (A). This secondary sperm cell morphological abnormality was predominant compared with the treated group. In conclusion, aqueous extract of *Spondia mombin* supported fertility in the male Wistar rat at concentration of 600mg/ml hence can be a good source of fertility enhancement in male animals.

Impact

There are a lot of reproductive problems currently. Some of these problems can be traced to habits in different societies especially the Third World countries. There are some other practices that are beneficial to reproduction but not discovered and documented. One such is the consumption of concoctions having large doses of *Spondias mombin*. This work was able to reveal a beneficial impact of this medicinal plant to the male reproductive indices.

Reference

Ayoka A O, Akomolafe R O, Akinsomisoye O S and Ukponmwan O E. 2008. «Medicinal and Economic Value of *Spondias mombin*» (PDF online reproduction). *African Journal of Biomedical Research* (Ibadan, Nigeria: Ibadan Biomedical Communications Group) 11: pp.129–136.

Ayoka A O, Akomolafe R O, Iwalewa E O, Akanmu M A and Ukponmwan O. E. 2006: Sedative, antiepileptic and antipsychotic effects of Spondias mombin L. (Anacardiacaea) in mice and rats. *Journal of Ethnopharmacology* 103, 166 – 175.

Ayoka A O, Akomolafe R O, Iwalewa E O and Ukponmwan O E. 2005: Studies on the anxiolytic effects of *Spondias mombin* L. (Anacardicaea) extracts. African Journal of Traditional, Complementary and Alternative Medicine 2 (2), 153 – 165.

Offiah V N and Anyanwu I I. 1989: Abortifacient activity of an aqueous extract of Spondias mombin leaves. *Journal of Ethnopharmacology* 26, 317 – 320.

Okaka J C, Enoch N J and Okaka N C. 1992: Human Nutrition : An integrated approach. ESUT Publications, Enugu, pp:57-58.

Oyeyemi M O, Towobola A B, Ola Davies E O 2006. The reproductive implications of Clomiphene citrate on sperm cells during the epididymal transit of spermatozoa in male wistar rat. Folia Veterinaria 50: 131-133

Raji Y, Gbadegesin M A, Osonuga O A, Adisa R A, Akinsomisoye O S, Awobajo, F O, Kunle-Alabi O T, Esegbue Peters P R C, Osonuga I O and Lamidi AF (2006)

Reproductive, *haematologic* and biochemical profiles of male rats treated with aqueous extract of Spondias mombin bark, *International Journl of Pharmacology* 2(1): 126-130

Sofowora, E.A. (1982): Medicinal plants and traditional medicines in Africa. John Wiley and Sons Ltd, Nigeria, pp 64-79

Steel RGD, Torrie JII (1996). Principles and procedure of Statistics. A biometric approach (2nd ed.), McGraw-Hill, New York, pp. 6-15.

Wilkinson J M S, Halley P A. Tower Comparison of male reproductive parameters in three rat strains: Dark Agouti, Sprague-Dawley and Wistar. 2000. Laboratory Animal; 34:70-75. - PudMed –Laboratory animal Limited

Evaluation of safety, growth promoting and immuno-potentiating activities of the African Baobab (*Adansonia digitata*) fruit-pulp extract in commercial broilers

Analyse des caractéristiques de sécurité, de promotion de la croissance et des potentialités immunisantes de l'extrait de pulpe du fruit de baobab africain (*Adansonia digitata*) pour la nutrition et le traitement des poulets de chair

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Abstract

Component parts of the African Baobab tree (*Adansonia digitata*) have been used for medicinal and nutritional purposes and standardized preparations derived from it have been reported to possess antiviral, anti-inflammatory and immune-boosting activities. This study was designed to investigate the safety, growth promoting and immune-boosting capacity of the fruit-pulp extract in vaccinated broilers. One hundred day-old broilers were separated into five groups of twenty per group. Infectious bursal disease (IBD) and Newcastle disease (ND) vaccines were administered orally to all groups on days 11 and 21 respectively. IBD vaccine booster dose was administered on day 25. Group A did not receive the fruit-pulp extract while groups B, C, D and E received 125mg, 250mg, 500mg and 1000mg/kg body weight respectively from 14 to 42 days of age in drinking water. Ten broilers were weighed and bled from each group weekly and sera harvested for haemagglutination inhibition (HI) and agar gel immuno-diffusion (AGID) tests up to 64 day-old to assess ND and IBD viruses antibody titers, respectively. Kidney and liver tissues were harvested at 9 week-old for histopathological examinations for lesions of toxicity. Significantly higher ($p<0.5$) mean weekly body weights was observed in groups B and C than in groups A, D and E from 5 to 9 week-old. Congested kidney vessels, generalized hepatic degeneration with focal areas of necrosis were observed in groups D and E. Although significant differences in ND virus antibody titers were observed between the groups at 21, 35 and 64 days of age and at 56 day-old for IBD virus antibody, no definite trend was observed throughout the experiment. This study has shown that the baobab fruit-pulp extract has no significant effect on antibody response of broilers to ND and IBD vaccinations but has growth promoting effect at 125 – 250mg/kg body weight per day without toxic effect.

Key words: African Baobab, Broilers, growth, *immune-potentiation*, safety

Résumé

La portée des présents travaux de recherche est d'étudier les caractéristiques évidentes de l'extrait de pulpe du fruit de baobab en matière de sécurité, de promotion de la croissance et de stimulation du système immunitaire des poulets de chair vaccinés. Une centaine de poussins dun jour ont été séparés en cinq groupes de vingt unités par groupe. Les vaccins contre la bursite infectieuse et la maladie de Newcastle ont été administrés par voie orale à tous les groupes, le 11ème et le 21ème jours respectivement. La dose de rappel du vaccin contre la bursite infectieuse a été administrée aux poussins le 25ème jour. Contrairement à ceux du groupe A qui nen ont pas reçu, les poussins des groupes B, C, D et E âgés de 14 à 42 jours ont respectivement reçu de l'extrait de

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pulpe du fruit de baobab dilué dans de l'eau potable à raison de 125 mg, 250 mg, 500 mg et 1000 mg par kg de poids corporel. Chaque semaine, dix poussins âgés au maximum de 64 jours sont tirés de chaque groupe et sont ensuite pesés et saignés en vue de recueillir des sérums destinés aux tests d'inhibition de l'hémagglutination (IH) et d'immuno-diffusion en gélose (IDG) aux fins d'évaluation des titres d'anticorps des virus de la maladie de Newcastle et de la bursite infectieuse, respectivement. Les tissus du rein et du foie ont été prélevés des poulets âgés de 9 semaines pour les examens histopathologiques des lésions dues à la toxicité.
Selon le constat effectué, les poids corporels moyens hebdomadaires des poulets de chair âgés de 5 à 9 semaines étaient nettement plus élevés ($p < 0,5$) dans les groupes B et C que dans les groupes A. L'on a relevé, chez les poulets des groupes D et E, la congestion des veines rénales, la dégénérescence hépatique généralisée avec des foyers de nécrose. Malgré les différences significatives enregistrées dans les titres danticorps du virus de Newcastle chez les groupes de poulets de chair âgés de 21, 35 et 64 jours, d'une part, et dans les titres d'anticorps du virus de la bursite infectieuse chez les poulets âgés de 56 jours d'autre part, aucune tendance claire na été observée pendant toute l'expérience. La présente étude semble confirmer que l'extrait de pulpe du fruit de baobab na pas deffet significatif sur la réponse immunitaire des poulets de chair par rapport aux vaccinations contre la maladie de Newcastle et contre la bursite infectieuse, mais qu'il a par contre une influence positive sur la promotion de leur croissance à raison de 125 à 250mg par kg de poids corporel par jour sans effet toxique.

Mots clés: Baobab d'Afrique, poulets de chair, croissance, stimulation du système immunitaire, sécurité.

Introduction

Ethno-veterinary medicine has been defined as the holistic, interdisciplinary study of local knowledge and its associated skills, practices, beliefs, practitioners, and social structures pertaining to the healthcare and good husbandry income-producing animals, always with a view to practical development applications within livestock production and livelihood systems, and with the ultimate goal of increasing human well-being via increased benefits from stock raising (McCorkle, 1986). Herbal therapy basically is the use of parts of plants to treat and prevent diseases. Herbs represent one of the first pharmacological interventions attempted by healers and in recent years medicinal plants have represented a primary source for the pharmaceutical industry (Ajose, 2007). In addition, more than 80% of the world's people, mostly in poor and less-developed countries, depend on traditional medicine for their primary health care requirements (Bajaj and Williams, 1995). Since traditional herbal therapeutics have proven efficacious by the standards of both historical and modern medicine, ethno-veterinary practices in Nigeria and elsewhere are used by animal owners and veterinarians to treat several disease conditions.

The poultry industry in Nigeria has become a popular enterprise for the small holders and has contributed greatly to the economy of the country. The industry has assumed greater importance in improving employment opportunities and animal food production in Nigeria with about 10% of the Nigerian population engaged in poultry production, mostly on subsistence and small or medium-sized farms (Okonkwo and Akubuo, 2001). In spite of her numerous human and natural resources, Nigeria still remains among the least consumers of animal protein in Africa (Egbunike, 1997). To increase protein intake in Nigeria, there is urgent need to increase broiler production at household level and in commercial holdings. Broiler production has a relatively short production cycle compared with other types of livestock and can easily bridge the gap in animal protein availability and consumption in Nigeria.

A major constraint to profitable

broiler production in the tropics is disease outbreaks. The tropical environment provides optimum conditions e.g. high temperature and humidity for disease agents to thrive. Such conditions as well as poor biosecurity are major challenges being faced by the poultry industry in Nigeria. It has therefore, become imperative to seek means of increasing resistance of poultry to diseases through ethno-veterinary practices.

The Baobab tree (*Adansonia digitata*) which is the most widespread of the *Adansonia* species on the African continent is found in the hot, dry savannahs of sub-Saharan Africa (Wickens, 1982; Sidibe and Williams, 2002). Different component parts of the Baobab tree have been used by many local farmers especially leaves, fruit-pulp, seeds and bark fibers, for medicinal and nutritional purposes (Sidibe and Williams, 2002; Chadare *et al.,* 2009) and some commercial enterprises also produce standardized preparations derived from seeds, fruit-pulp and leaves which have been reported to possess antiviral, anti-inflammatory and immune-boosting activities (Anani *et al.,* 2000, Vimalanathan and Hudson 2009). In addition, antibacterial and anti-trypanosome activities have been reported (Hudson *et al.,* 2000; Atawodi *et al.,* 2003). The probable immuno potentiating effect in chickens has not received sufficient attention (El-Rawy *et al.,* 1997).

This study was, therefore, carried out to ascertain the safety, growth promoting and and immuno-potentiating activities of the fruit-pulp extract on commercial broilers vaccinated with Newcastle disease (ND) and infectious bursal disease (IBD) vaccines.

Materials and Methods

Extract Preparation

The aqueous cold extraction method was used. Twenty-five grams of Baobab fruit-pulp was soaked in 200 ml of distilled water for 48 hours in a plastic jar. This suspension was filtered and the filtrate, containing 125mg of extract per ml was administered to the birds in drinking water daily (based on the group weight) according to the treatment plan.

Rearing and Medication of Broilers

One hundred day-old broiler chicks (Arbor acre) were purchased from a local hatchery in Ibadan, Nigeria and were raised in the Experimental animal unit of the Department of Veterinary Medicine, University of Ibadan for two weeks after which they were transferred to the Broiler Production unit of the University of Ibadan Teaching and Research Farm. All rules and regulations of the University of Ibadan governing the use of experimental animals were followed. The broilers were given glucose on the first day of life as well as Floxatril® (Norfloxacin) and multivitamins in drinking water within the first week of life. After the first week, the broilers were randomly divided into five groups of twenty birds each and were tagged A, B, C, D and E. Group A was the unmedicated control group, group B was administered 125mg/kg body weight of *A.digitata*, fruit-pulp extract, group C was administered 250 mg\kg, group D was administered 500mg/kg while group E was administered 1000mg/kg. The broilers were dosed daily via drinking water for 4 weeks from 14 to 42 days of age and ten out of twenty birds in each group were weighed weekly.

Vaccination and Sample Collection

All the broilers were administered infectious bursal disease (IBD) and Newcastle disease (LaSota strain) vaccines (National Veterinary Research Institute, Vom, Nigeria) orally at 11 and 21 days old respectively. Each vaccine vial was diluted in 10 mls of phosphate buffered saline (PBS) and made up to 1 liter after which the broilers were served at a dose of 10 mls per bird. IBD vaccine booster dose was administered at 25 day-old. The broilers were bled for serology at 14, 21,

35, 42, 56 and 64 days of age via the brachial vein. Two mls of blood were collected into plain bijou bottles and kept at room temperature for serum to exude. Serum was then decanted into eppendorf tubes for analysis by haemagglutination inhibition (HI) and quantitative agar gel immuno-diffusion (AGID) tests for the assessment of ND and IBD viruses antibody titers respectively.

Haemagglutination-Inhibition Test

The β procedure of haemagglutination-inhibition test was carried out on serum samples harvested from the broilers. The technique used was a slight modification of the method described by Thayer and Beard (1998). A multi-channel pipette was used to dispense 25 µl of PBS into all the wells of a 96 well U-bottom *microtitre* plate. Twenty-five µl of serum sample was then added into the first well of each row and this was serially diluted. Twenty-five µl of 4HA units ND virus antigen was added to each well. The plate was shaken with a *microtitre* plate shaker and incubated at room temperature for 30 minutes. Twenty-five µl washed 1% chicken RBC was added to each well and the plate was again incubated for 30 minutes after which each plate was tilted to observe for "tear dropping" which shows that erythrocytes were not agglutinated i.e. haemagglutination inhibition. The reciprocal of the highest dilution of serum at which there was complete inhibition of heamagglutination was recorded as the titer of ND virus antibody in the serum.

Quantitative Agar Gel Immuno-diffusion (AGID) Test

The *quantitative Agar gel* immuno-diffusion technique used was as described by Cullen and Wyeth (1975). One gram of Agarose and 8 gm of NaCl were measured into a Duran bottle and 100 mls of distilled water was added. The bottle and its content were autoclaved for 15 minutes. The molten agar was dispensed into 100-mm disposable petri dishes at the rate of 15 mls per dish and was allowed to set. Using a template, seven holes were bored into the gel (six at the periphery, one at the centre) to form a ring. Each plate was punched to contain twelve rings.

Using a multi-channel pipette and U-bottom microtitre plates, 50µl of each of the collected serum was serially diluted (double-fold) with PBS to the tenth well

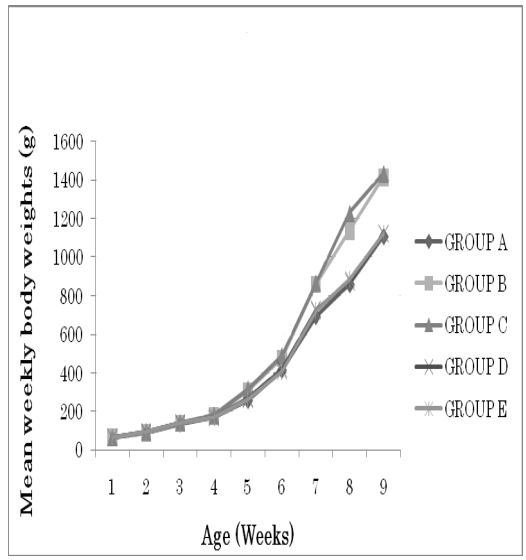


Figure 1: Average weekly body weights of broilers administered fruit pulp extract of *A.digitata*

i.e. 1:1024 dilution. From each well, diluted serum was pipetted into a peripheral well. Five wells were loaded per ring while the sixth well contained IBD virus antibody positive control serum. IBD virus antigen was loaded into the central well and the plate incubated at 37°C for 24 hours. The reciprocal of the highest dilution at which a line of precipitation occurred for each serum sample was recorded as the IBD virus antibody titer of the serum sample.

Histopathology

At 9 weeks of age, tissues from liver and kidneys of three broiler chickens from each of the five groups were harvested and fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely

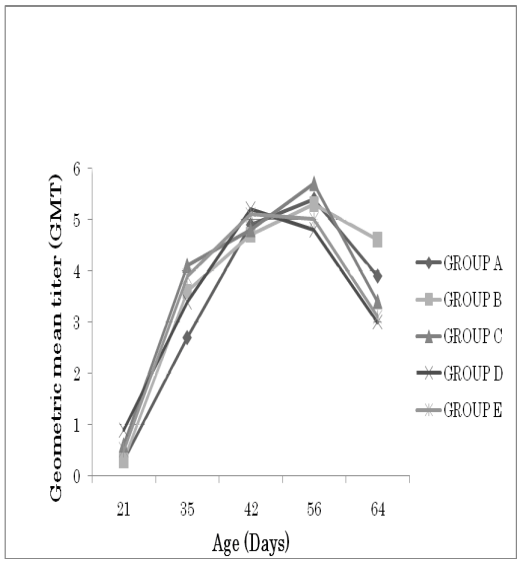


Figure 2: Newcastle disease antibody response of vaccinated broilers administered fruit pulp extract of A digitata

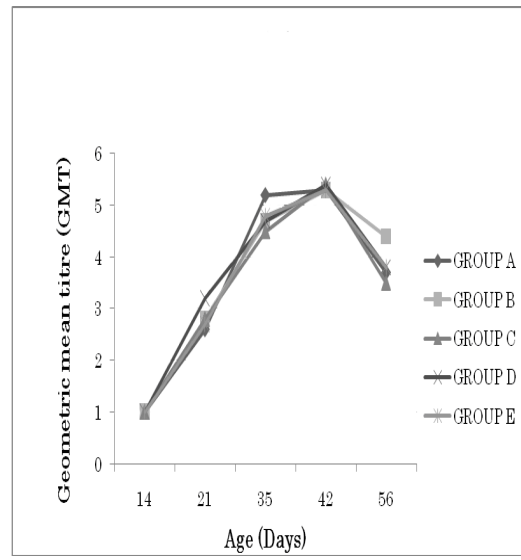


Figure 3: Infectious bursal disease antibody responce in vaccinated broilers administered fruit pulp extract of A digitata

and embedded in paraffin wax. Sections of 5 µm thickness were cut, stained with haematoxylin and eosin and examined under the light microscope.

Statistical Analysis

Results obtained from the HI and quantitative AGID tests as well as weekly body weights measured were subjected to statistical analysis using One way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). These were used to determine if there were significant differences in mean values between the five groups of broilers.

Results

Average weekly body weights for the five groups increased steadily from week 1 and were similar from week 1 to week 4. However, body weights from week 5 to week 9 were significantly higher ($P<0.05$) in groups B and C than in groups A, D and E (Figure 1).

The results of the Newcastle disease HI tests conducted on serum samples obtained from the five groups of broilers showed that the trend of antibody response to ND vaccination was similar in the five groups. ND antibody levels consistently increased in the five groups from geometric mean titers (GMT) of less than 1.0 at 21 days of age, peaked at about 5.0 at 56 days of age and declined to values between 3.0 and 4.6 at 64 days of age. At 21 days of age, broilers in group D had significantly higher GMT than groups A and B ($P<0.05$). At 35 days of age, group A broilers had significantly lower GMT ($P<0.05$) than groups B, C and E while at 64 days of age, broilers in groups C, D and E had significantly lower ($P<0.05$) GMT than groups A and B (Figure 2).

Agar gel precipitation test conducted on serum samples obtained from the five groups of broilers showed that the trend of antibody response to IBD vaccination was similar in the five groups. IBD antibody levels consistently increased from GMTs ranging between 2.6 and 3.2 in all 5 groups at 21 days of age peaked at values ranging from 5.3 to 5.4 at 42 days old and declined to values between 3.5 and 4.4 at 56 days old. Differences obtained between the GMTs for the five groups were statistically significant only at 56 days of age

($P<0.05$) where the GMT of group B was significantly higher than the other groups (Fig. 3).

Histopathological examination of tissues from the five groups of broilers revealed varying degrees of dilation of hepatic sinusoids in groups B and C. In groups D and E, kidney vessels were slightly congested and there was diffused hepatic degeneration with focal areas of necrosis. However, liver and kidney tissues from broilers in group A showed no significant lesion.

Discussion

This study has evaluated the potential of the fruit-pulp extract of the African Baobab (*A. digitata*) as a growth promoter and an immune booster in broilers. Antibody responses to IBD and ND vaccinations were used as indicators for immune boosting activity while weekly body weight was used to assess growth promoting effect in broilers.

Mean weekly body weights for all groups did not differ appreciably until the fifth week from when groups B and C had consistently higher values than those of groups A, D and E until the 9th week when the experiment was terminated. This shows that the fruit-pulp extract of *A. digitata* has growth promoting effect which is dose-dependent. According to Lockett *et al.*(2000), the fruit-pulp extract of Baobab tree has 5.3g protein per 100g of extract comprising major amino acid necessary for growth. However, groups B and C with higher mean body weights received lower doses of the extract (i.e. 125mg/kg and 250mg/kg body weights, respectively) than groups D and E (500mg/kg and 1000mg/kg body weights, respectively). Thus, it appears the optimum dose of the pulp extract for improved growth was between 125mg and 250 mg/kg body weight. The histopathological lesions of hepatic degeneration with focal areas of necrosis as well as kidney congestion observed in groups D and E are suggestive of hepato-toxicity. Since these lesions were not observed in the groups with lower doses (groups B and C) and in the untreated group, it therefore implies that beyond 250mg//kg body weight, the extract probably becomes toxic. Al-Qarawi *et al.* (2003) had earlier reported hepatoprotective effect of the extract in rats at 1mg/kg body weight. The growth promoting effect of the fruit-pulp extract at 125mg/kg and 250mg/kg could be ascribed to the presence of several amino acids, minerals, vitamins and sugars as reported by Lockett *et al.* (2000) and Gebauer *et al.* (2002).

Increased ND antibody titres were observed in all groups from 35 days of age up to 56 days after which a decline was observed. The observed trend is the result of ND vaccinations at day-old and at 21 days of age. According to Alexander (1991), antibody levels increase rapidly after two weeks of vaccination in healthy chickens. With regards to antibody response to IBD vaccination, the trend is similar to that of ND antibody titers obtained in the broilers although peak titers in all the groups were observed earlier, i.e. at 42 day-old. The results showed that administration of the fruit-pulp extract of *A.digitata* to broilers at 125 – 1000mg/kg body weight has no significant advantage on antibody response to ND and IBD vaccinations which is contrary to the reports of Manfredini (2000) and El-Rawy *et al*. (1997). The report of that Vitamin C stimulates antibody production was not also substantiated in this study as *A.digitata* extract was reported to contain 300mg of Vitamin C/100g of pulp extract (Eichelbaum, 1996, Orata and Ondachi 2001). However, the absence of influence on antibody production contrary to the observation of earlier studies might be due to the low dosage of vitamin C in the extract dosages used in this study

In conclusion, this work has shown that the fruit-pulp extract may not have effect on antibody response to ND and IBD vaccinations in broilers but could be used as a natural growth promoter in

broiler production in the tropics at specific dosage.

Impact

Component parts of the African Baobab tree (*Adansonia digitata*) have been used for medicinal and nutritional purposes and standardized preparations derived from it have been reported to possess antiviral, anti-inflammatory and immune-boosting activities.This study has shown that the fruit-pulp extract has growth-promoting effect between 125mg and 250mg/kg body weight without *toxic* effect. Dosages above 250mg/kg body weight were found to be toxic to the boilers. However, the immune-boosting activity could not be confirmed via antibody response to ND and IBD vaccinations.

References

Ajose FAO, 2007. Some Nigerian plants of *pharmacological* importance. *International Journal of Dermatology,* 46 (Supplement1): 48-55.

Alexander DJ, 1991. Newcastle disease and other *paramyxovirus* infections. In: Diseases of poultry, Eds., Calnek BW, Barnes HJ, Beard CW, Reid WM and Yoder Jr., HW 9th edn: .*Iowa State University Press,* Ames, Iowa, USA, pp: 496-519.

Al-Qarawi AA,Al-Damegh MA and El-Mougy SA, 2003. Hepatoprotective influence of Adansonia digitata pulp. *Journal of Herbs, Spices & Medicinal Plants,* 10(3): 1-6.

Anani K, Hudson JB, de Souza C, Akpagana K, Towers GHN, Arnason JT and Gbeassor M. 2000.*Investigation of Medicinal Plants of Togo for Antiviral and Antimicrobial Activities. Pharmaceutical.* Biology, 38: 40-45.

Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman N and Galadima M. 2003. In vitro *trypanocidal* effect of methanolic extract of some Nigerian savannah plants.African *Journal of Biotechnology*, 2: 317-321.

Bajaj M and Williams JT, 1995. Healing forests healing people. IDRC Medicinal Plants Network, New Delhi, India. Bsoul SA, Terezhalmy GT, 2004.Vitamin C in health and disease. *Journal of Contemporary Dental Paractice,* 5(2): 1-13.

Chadare FJ, Linnemann AR, Hounhouigan JD, Nout MJR and Van Boekel MAJ, 2009. Baobab food products: A review on their composition and nutritional value. Critical Reviews in Food Science and Nutrition,49: 254-274.

Cullen GA and Wyeth PJ, 1975. Quantitation of antibodies to infectious bursal disease. *Veterinary Record,* 97:315.

Egbunike GN. 1997. What is animal science? And how can Nigeria get out of malnutrition. In: Livestock products, Eds., Ologhobo, AD, Iyayi, EA, Adesehinwa, AOK, Bamgbose AM: Proceedings 2nd Annual Conference of *Animal Science Association of Nigeria.* pp. 1-12.

Eichelbaum M and Gross S. 1996. Anti-inflammatory, analgesic and *antipyretic* effects of the fruit pulp of *Adansonia digitata. Fitoterapia*, 65(5): 418-422.

El-Rawy, Eman M, Gergis SM, Bazaid S and ElMougy S.A, 1997. The immunostimulant effect of *Adansonia digitata* on the immune response of chicken vaccinated with *avian cholera* vaccine. *Journal of Egyptian Veterinary Medical Association,* 57: 959-970.

Gebauer J, El-Siddig K and Elbert G, 2002. Baobab (Adansonia digitata L) a review on a multipurpose tree with promising future in Sudan. Gartenbauwissenschaft 67 (4) S:155-160.

Hudson JB,Anani K, Lee MK, De Souza C,Arnason JT and Gbeassor M. 2000. Further investigations on the antiviral properties of medicinal plants of Togo. *Pharmaceutical Biology* 38: 46-50.

Lockett CT, Calvert CC and d Grivetti LE. 2000. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, North-eastern Nigeria. *International Journal of Food Science Nutrition,* 51: 195-208.

Manfredini S, 2002. The health properties of Baobab (Adansonia digitata). Postgraduate

seminar presentation, *University of Ferrara, Verona.* pp. 23-27.

McCorkle CM, 1986. An introduction to Ethnoveterinary research and development. *Journal of Ethno-biology,* 6(1): 129-149.

Okonkwo WI and Akubuo, CO, 2001. Thermal analysis and evaluation of heat requirement of a passive solar energy poultry chick brooder in Nigeria. *Journal of Renewal Energy,* 9: 1.

Orata D and Ondachi P W 2001. Enhanced electrodetection of ascorbic acid in *Adansonia digitata* fruit by use of surface modified electrodes. *Catalysis Letters,* 72(1-2): 125-128.

Sidibe M and Williams JT 2002. Baobab. Adansonia digitata. International Centre for Underutilized Crops. *University of Southampton*. ISBN 0854327762.

Thayer SG and Beard CW 1998. Serological procedures. In: A laboratory manual for the isolation and identification of *avian pathogens*. 4th edn. Eds.., Swayne DE, Glisson JR., Jackwood MW, Pearson JE, Reed WM 4th edn. pp. 255-266. *American Association of Avian Pathologists,* University of Pennsylvania, Kennett Square, PA.USA. pp255-266.

Wickens GE, 1982. The baobab – Africa's upside-down tree. *Kew Bulletin.,* 37: 173-209.

Vimalanathan S and Hudson JB. 2009. Multiple inflammatory and antiviral activities in Adansonia digitata (Baobab) leaves, fruits and seeds. *Journal of Medicinal Plants Research,* 3(8): 576-582.

Effect of diets supplemented with garlic essential oil and streptomycin sulphate on intestinal microflora and nutrients digestibility of broilers

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Abstact

An experiment was carried out to study the comparative effects of garlic essential oil and streptomycin sulphate on intestinal microflora and nutrients digestibility of broilers. Forty eight Hubbard line day old chicks with equal numbers of males and females were randomly divided into four treatments to conduct a 4x2 factorial experiment in a completely randomised design. The diets were: no supplements (control), garlic essential oil at 40 ppm/kg (GEO 40ppm), garlic essential oil at 60 ppm/kg (GEO 60ppm) and streptomycin sulphate at 30 ppm/kg (SS 30ppm) administered by oral gavage from day13 to day 47 of experiment. There were two birds (males or females) per experimental unit, replicated three times in twenty four deep litter pens. The colony forming units of *Escherichia coli* were significantly lower ($P<0.001$) in the ileo-caecal digesta of birds on streptomycin sulphate (3.33 x 105) followed by the garlic essential oil treated groups ($\geq 4.08 \times 10^5$) compared with the control (8.50×10^5). The same observation was made for *Staphylococcus aureus* ($P<0.001$). The colonies of *Salmonella* and *Shigella spp* were statistically similar between *streptomycin sulphate* and garlic essential oil treated groups ($\geq 1.65 \times 10^5$), but they were significantly ($P<0.001$) reduced compared with the values obtained in the control group (4.53×10^5). Female broilers had higher ($P<0.001$) colony forming units of *enterobacteriaceae, Salmonella* and *Shigella spp* and *Staphylococcus aureus* in their ileo cecal digesta than the males. Even within the treatment and sex interaction, female birds generally recorded higher number of colony forming units as compared with the males. Only mold fungi were found in the ileo-cæcal digesta of all the groups. Significant improvement in apparent digestibility of nutrients except for the calcium and inorganic phosphorus absorption rate in birds on supplemented diets was observed ($P<0.01$) compared with those on the control. There were no significant differences ($P>0.05$) in nutrients absorption between male and female broilers. Treatment and sex interaction significantly ($P<0.05$) affected all the parameters studied indicating a synergistic effect of the two factors on nutrients absorption. In conclusion, GEO even at 40 ppm/kg controlled pathogens and improved nutrients digestibility in birds.

Keywords: garlic essential oil, ileo cecal digesta, intestinal microflora, nutrients digestibility, streptomycin sulphate.

Résumé

Une expérience a été réalisée pour étudier les effets comparatifs de l'huile essentielle d'ail et de sulfate de streptomycine sur la microflore intestinale et la digestibilité des nutriments chez les poulets de chair. Quarante-huit poussins Hubbard d'un jour en nombre égal de mâles et femelles ont été repartis au hasard en quatre traitements pour mener une expérience dans un dispositif factorielle 4x2 complètements aléatoires. Les traitements étaient faits de : pas de suppléments

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(contrôle), l'huile d'ail essentiels à 40 ppm / kg (GEO 40ppm) et à 60 ppm / kg (GEO 60ppm) et de sulfate de streptomycine à 30 ppm / kg (SS 30 ppm), administré par gavage oral du jour 13 au jour 47.

Les colonies d'Escherichia coli ont été significativement plus faible (P <0,001) dans le digesta iléo-caecal des oiseaux recevant du sulfate de streptomycine (3,33 x 105) suivi par ceux recevant l' ail, huile essentielle et traités (≥ 4,08 x 105) et le groupe contrôle (8,50 x 105). La même observation a été faite pour le Staphylococcus aureus (P <0,001). Les colonies de Salmonella et Shigella étaient statistiquement similaires entre les animaux recevant du sulfate de streptomycine et ceux recevant de l'd'huile essentielle d'ail (≥ 1,65 x 105), mais ils étaient significativement (P <0,001) réduite par rapport aux valeurs obtenues dans le groupe témoin (4,53 x 105). Les colonies d'entérobactéries, Staphylococcus Salmonella et Shigella et Staphylococcus dans leur digesta caecale iléo étaient plus élevé chez les poulets femelles (P <0,001) comparees aux mâles. Une amélioration significative de la digestibilité apparente des nutriments a été observée (P <0,01) chez les animaux recevant des supplements compares au contrôle sauf pour le calcium et le phosphore inorganique. Il n'y avait pas de différences significatives (P> 0,05) entre les sexes. L'interaction traitement et le sexe a été significative (P <0,05) sur tous les paramètres étudiés ce qui indique un effet synergique des deux facteurs sur l'absorption des nutriments. En conclusion, GEO, même à 40 ppm / kg permet de contrôler les pathogènes et d'améliorer la digestibilité des nutriments chez les oiseaux.

Mots-clés: ail, huile essentielle, iléo caecale digesta, la microflore intestinale, la digestibilité des nutriments, de sulfate de streptomycine.

Introduction

The strongest determinant of the gut microbial profile is the hosts diet. Factors such as diet composition, nutrient concentration, feed physical traits, feed processing, and feed additives play significant roles in the dynamics of gut microflora (Apajalahti *et al.,* 2001, 2004; Guo *et al.*, 2004, Hume *et al.,* 2003, 2006; Oviedo-Rondón *et al*., 2006; Parker *et al.,* 2007, Nalian *et al*., 2009). Microbes have profound effects in some of the physiological processes of their animal host (Ewing and Cole, 1994; Fuller and Perdigon, 2003). Digestive microflora populations affect broiler and layer hen performance and health (Apajalahti and Bedford, 1999; Hume *et al.,* 2003, 2006; Oviedo-Rondón *et al*., 2006; Parker *et al*., 2007). These effects in the host may be due primarily to the complex interactions that influence the intestinal environment, the development and responses of the host immune system against pathogenic and non-pathogenic antigens (Cebra, 1999). Essential oils derived from herbs have been shown to have antimicrobial effects (Dorman and Deans, 2000). Their antimicrobial mode of action consists of interactions with the cell membranes of micro-organisms by changing permeability for cations such as H+ and K+ (Ultee *et al.,* 1999). Moreover, there are evidences that herbs, spices and various plant extracts have appetizing, digestion-stimulating properties and antimicrobial effects (Gill, 1999; Langhout, 2000; Madrid *et al.,* 2003; Alçiçek *et al*., 2004; Zhang *et al*., 2005). The improvement in feed efficiency achieved with essential oil mixtures could be attributed to their positive effects on nutrients digestibility (Langhout, 2000; Madrid *et al.,* 2003; Hernandez *et al.,* 2004; Jamroz *et al,.* 2005; Garcia *et al.,* 2007 and Loh *et al.,* 2008). This study was conducted to determine the effects of garlic essential oil and streptomycin sulphate on gut microflora and nutrients digestibility in broilers.

Materials and Methods

Animals and experimental design

Forty eight day old chicks of Hubbard line with equal numbers of males and females were selected from the batch kept in a brooding room of Abubakar Tafawa Balewa University Poultry Research Farm, Bauchi state, Nigeria for two weeks and transferred to experimental pens. The

brooding room temperature decreased from 32°C during the first week of life to 28°C in the second. In order to boost their immunity, they were vaccinated against Infectious bursal disease on the 14th day of the experiment while Newcastle disease vaccine was administered at 21days of age. Experimental diets and water were given to birds *ad libitum* every day. The entire flock was subject to deworming on the 35th day of age using piperazine. The experiment lasted for five weeks during which feed intake, weekly weight gain and feed conversion ratio were monitored. Two broilers died of

Table 1: Composition of experimental starter and finisher diets

Ingredients	Percent starter	Percent finisher
Maize (7.6.% CP)	51.25	56.80
Rice bran (11.8% CP)	08.00	10.00
Soybean meal (44% CP)	33.00	30.00
Fish meal (72% CP)	3.50	------
Bone meal	2.50	2.50
Vitamin/Mineral Premixk (0.25%)	0.25	0.25
Sodium chloride (NaCl)	0.25	0.25
Methionine(99%)	0.20	0.20
Totals in kilogramme	100.00	100.00
Feed nutrients proximate analysis		
Metabolizable Energy (Kcal/kg)	2818.33	2855.37
Crude protein (%)	22.05	19.08
Crude fibre (%)	4.21	4.31
Fats (%)	4.58	6.31
Calcium (%)	1.02	1.00
Available Phosphorus (%)	0.49	0.47
Lysine (%)	1.25	1.03
Methionine (%)	0.58	0.51

keach 2.5kg premix contained the followings:
Vit A 10,000,000 IU;Vit. D3 3,000,000 IU;Vit. E 30,000 IU;Vitamin K 2 3 g; Vit B11.7g;Vit B2 5.0 g;VitB6 3.1g;Vit. B12 16 mg; Biotin 60 mg; Niacin 1.0 g; Pantothenic Acid 8 g; Folic Acid 0.8 g; Manganese 85 g; Zinc 50 g; Iron 25 g; Copper 6 g; Iodine 1.1 g; Selenium 120 mg; Cobalt 220 mg; B.H.T 60 g; Ethoxyquin 65 g; Choline Chloride 200 g

Table 2: Chemical composition (%) of Allium sativum oil

No	Retention Index	Compound Name	Percent in oil
		Thioethers	**83.67%**
1	660	Allyl methyl sulfide	3.49
2	849	1-propene, 3,3'-thiobis-sulfide	3.99
3	1099	Disulfide, di-2-propenyl	9.78
4	1131	Trisulfide, methyl 2-propenyl	26.82
5	1134	3-vinyl-1,2-dithiacyclohex-5-ene	32.72
6	1350	Trisulfide, di-2-propenyl	6.86
		Fatty acids	16.33%
7	1968	n-hexadecanoic acid	6.51
8	2183	Linoleic acid	9.82

coccidiosis in the fourth week of experiment giving a percentage mortality of 4.16% and the whole flock was thereby subject to five days of cure with pure amprolium.

The forty eight chicks were randomly divided into four (4) treatments to conduct a 4x2 factorial experiment in a completely randomised design as follows: Control (Water), Garlic oil 40mg/kg/day, Garlic oil 60mg/kg/day, Streptomycin sulphate 30mg/kg/day (Fraser *et al*., 1991; Radostits *et al,* 1997; Group Zhongnuo Pharmaceutical (shijiazhuang) Co.,Ltd.) administered by oral intubation from day13 to day 47 of the experiment. There were two birds (males or females) per experimental unit, replicated three times in twenty four (24) deep litter pens. Weekly weighings of birds were carried out to determine the concentration of treatments to be given to birds. The quantity of garlic oil and streptomycin sulphate administered were calculated taking into account the proportion of the major oil component, the minimum recommended oral route dose of the antibiotic and the chicken live weights.

Diets and feeding regimens

Birds were fed with commercial starter and finisher diets (Animal Care Services Konsult Nig. Ltd.) formulated to meet their nutrient requirements through out the experiment (Table 1). As for the supplementation, garlic essential oil was obtained by organic solvent extraction (Soxhlet,1879) and oil analysis was carried out using Adams (2001) method (Table 2).

Garlic oil and streptomycin sulphate control on gut pathogens

Four males and four females from each treatment were randomly selected, weighted and slaughtered. Faecal samples collected from the ileo-caecal junction of the 32 eviscerated birds on the farm were put into sterilised vials and conveyed immediately to the laboratory. Then 1g of digesta taken from each sample was added to 10ml sterile distilled water and mixed for one minute in test tube. A tenfold serial dilution was made. Finally, 0.1ml was pipetted from the 1/1000 dilution test tube of each sample and inoculated on the solid culture medium prepared in Petri dishes the previous day. Dispersion was done using a sterile spreader sterilised after each step over a bunsen flame.

Yeast and mold fungi were cultured on Sabouraud agar medium mixed with 250mg chloramphenicol in order to inhibit any bacterial growth. They were incubated at 37°C for 24 hours and kept on the media preparation bench up to two weeks for identification. Bacterial counts were performed using *Salmonella/Shigella* agar medium for *Salmonella* and *Shigella* species, MacConkey agar for *Escherichia coli*, then the medium for identification of *Staphylococci spp* was prepared using Nutrient Agar + 12% (w/v) dilution of sodium chloride (NaCl). They were all subjected to incubation at 37°C for 24 hours (Johnston and Booth, 1983; Sinclair and Dhingra, 1995).

Digestibility studies and proximate analyses of feed and dried faecal collections

Four males and four females per treatment were randomly selected and subjected to digestibility study in battery cages four five (5) days. Birds were all fed with the commercial broiler finisher diet. On the first day of digestibility study in battery cages, birds were given only the supplementations in the morning and without feed till 3.00pm. Materials for fecal collections were placed under the cages in the morning of day 2 and wet faecal collections started on the third day in the morning before feed distribution coupled with oral supplementations. Dryed matter digestibility was calculated for each sample. The sun-dried faecal collections and the basal diet as well were subject to laboratory analyses to determine their contents in crude protein, crude fibre, and ether extract according to the AOAC (1995) procedures. Calcium and inorganic phosphorus were analyzed by UV absorption spectrophotometry [(Atomic

Table 3: Effects of garlic essential oil and streptomycin sulphate supplementations on the gut microbiota expressed in colony forming units per gramme of ileo-cecal digesta of broilers

Parameters	Control	Garlic Essential Oil		Streptomycin	SEM	P value
		40 ppm	60 ppm	30 ppm		
Staphylococcus aureus x 10^5	8.36[a]	5.51[b]	4.76[c]	4.51[c]	24138.75	0.000
Salmonella & shigella spp x 10^5	4.53[a]	2.28[b]	1.65[b]	2.70[b]	42561.27	0.000
Escherichia coli x 10^5	8.50[a]	4.97[b]	4.08[c]	3.33[d]	23433.36	0.000
Presence/absence of Yeast & Mold fungi	Mold present	Mold present	Mold present	Mold present		

Absorption Electronic Machine, Shimadzu, UV-1201, Japan)].

Statistical analysis

The data collected were compared using the analysis of variance (ANOVA) option of Minitab (version 11.0) and Compare Means option of Statistical Package for Social Sciences software (version 11.0) as described by Steel and Torrie (1980). Significantly different means among treatments were separated using the Duncan's Multiple Range Test (Duncan, 1955) at ($P<0.05$).

Results

Effects of garlic oil and streptomycin sulphate supplementations on intestinal microflora of chickens

The intestinal microbial counts in ileo-caecal digesta of broilers are given in Table 3. Birds in the control group had the highest number of colony forming units (CFU) of enteropathogens studied as compared with those on garlic oil and *streptomycin* sulphate. it was also observed that these colonies reduced as the dosage of garlic oil increased and the drop in CFU was even better with streptomycin sulphate except for *Salmonella* and *shigella spp*. Only mold fungi were observed in the ileo-cecal digesta of all the groups.

Sex effects of garlic oil and streptomycin sulphate supplementations on gut microbial population of broilers

The sex effects of garlic oil and streptomycin sulphate supplementations on gut microbiota of broilers are shown in Table 4. Female broilers had higher ($P<0.001$) colony forming units of *Escherichia coli*, *Salmonella* and *Shigella spp* and *Staphylococcus aureus* in their ileo cecal digesta than the males. However, the effect of treatments significantly decreased ($P<0.001$) the mean values of colony forming units of these enteropathogens per gramme of digesta collected from males as compared with those observed in female boilers. Mold fungi alone were found in the digesta of female birds and the males as well.

The treatment and sex interaction (Table 5) also significantly affected ($P<0.01$) the count of *Staphylococcus aureus*, the counts of *Salmonella* and *shigella spp*, and that of *E.coli* ($P<0.001$) per gramme of digesta studied indicating that the two factors

Table 4: Sex effects of garlic essential oil and *streptomycin sulphate* supplementations on gut microbial counts in broilers

Parameters	Male	Female	SEM	P value
Staphylococcus aureus x 10^5	5.17	6.40	4217.78	0.000
Salmonella and Shigella species x 10^5	2.67	2.91	2645.13	0.000
Escherichia coli x 10^5	5.01	5.43	2242.17	0.000
Presence/absence of Mold &Yeast fungi	Mold (+)	Mold (+)		

Table 5: Treatment x Sex interaction of garlic essential oil and *streptomycin sulphate* supplementations on gut *microflora* of broilers

Parameters	Control		GEO 40ppm		GEO 60ppm		SS 30ppm		SEM	P value
	Male	Female	Male	Female	Male	Female	Male	Female		
Staphylococcus aureus x 10^5	7.81^{b}	8.91^{a}	4.90^{f}	6.12^{c}	3.95^{h}	5.58^{d}	4.03^{gh}	5.00^{ef}	8435.57	0.004
Salmonella & Shigella spp x 10^5	4.50^{a}	4.57^{a}	2.25^{e}	2.32^{ed}	1.30^{g}	2.00^{f}	2.65c	2.75^{cb}	5290.27	0.000
Escherichia coli x 10^5	8.00^{b}	9.00^{a}	4.75^{d}	5.20^{c}	4.00^{f}	4.17^{fe}	3.32^{h}	3.35^{hg}	4484.34	0.000
Yeast and Mold fungi	Mold present	Mold present	Mold present	Mold present						

^{a,b,c,d,e,f,g,h} Mean values in the same row with different superscripts are significantly different.
*GEO: Garlic Essential Oil ; SS: Streptomycin Sulphate

Table 6: Treatments effect of garlic essential oil and *streptomycin* sulphate supplementations on nutrients absorption

Parameters	Control	Garlic Essential Oil		Streptomycin	SEM	P value
		40 ppm	60 ppm	30 ppm		
*ADDM	60.49^{b}	72.96^{a}	72.07^{a}	70.26^{a}	1.499	0.003
ADCP	51.48^{b}	65.61^{a}	64.91^{a}	60.80^{a}	1.843	0.001
ADCF	2.45^{b}	17.34^{a}	14.94^{a}	15.01^{a}	1.990	0.022
ADEE	90.28^{b}	93.42^{a}	93.10^{a}	92.11^{a}	0.365	0.044
CaAR	19.28^{b}	45.43^{a}	42.82^{a}	32.74^{ab}	3.023	0.034
PiAR	3.46^{b}	18.62^{a}	10.65^{ab}	7.11^{b}	1.890	0.020

^{a,b} Mean values in the same row with different superscripts are significantly different.
*ADDM: Apparent digestibility of dry matter, ADCP : Apparent digestibility of crude protein, ADCF : Apparent digestibility of crude fibre
ADEE: Apparent digestibility of ether extract, Ca AR: Calcium Absorption Rate, Pi AR : Inorganic phosphorus Absorption Rate

contributed synergistically to the decrease in number of these parasites in broiler chickens. Even within the treatment and sex interaction, female birds generally recorded higher number of colony forming units as compared with the males. Only mold fungi were found in the ileo-cecal digesta of all the groups.

Effects of garlic oil and streptomycin sulphate supplementations on nutrients digestibility

Treatments effect of garlic oil and streptomycin sulphate supplementations on nutrients absorption

Table 6 presents the results of nutrients digestibility in broilers subjected

to garlic oil and streptomycin sulphate supplementations and they were all significantly affected by the treatments. The values of apparent digestibility of nutrients in birds on control were all lower than those obtained from birds on supplemented diets except for the calcium absorption rate in birds on diets fortified with streptomycin sulphate and the inorganic phosphorus absorption rate in birds on diets including garlic oil 60ppm and streptomycin sulphate.

Sex effects on nutrients digestibility

The results of sex effects on nutrients digestibility in broilers subjected to garlic oil and streptomycin sulphate supplementations showed no significant differences (P>0.05) between male and female broilers and they are shown in Table 7. However, the mean values of nutrients digestibility in female birds were slightly superior to those of the males for the parameters studied.

Treatment and sex interaction (Table 8) on nutrients digestibility significantly (P<0.05) affected all the parameters studied indicating a synergistic effect of the two factors on nutrients absorption. Even within the interaction, the digestibility of nutrients in female birds were generally higher when compared with the values in males for all the parameters studied except for those on diets fortified with streptomycin sulphate.

Discussion

The effects of garlic oil and streptomycin sulphate supplementations on intestinal microbial population of broiler chickens showed that the number of colony forming units of *Staphylococcus aureus* per gramme of digesta from birds in the control group was significantly higher (P<0.001) than the value found in birds on garlic oil 40ppm which in turn was significantly higher (P<0.001) than those from birds on garlic oil 60ppm and streptomycin sulphate. Likewise, Guo (2003) used mushroom and herb polysaccharides as alternative for antimicrobial growth promoters in poultry and observed alteration of gut microbial activities and composition of chikens' caeca. The highest number of colony forming units (CFU) of *Escherichia coli* in ileo-cecal digesta was found in birds on the control and the lowest number in birds on streptomycin sulphate and they were all significantly different (P<0.001) from one another even as the dosage of garlic oil used increased. Likewise, Juneja and Friedman (2007) in an in vitro study using carvacrol, cinnamaldehyde, oregano oil, and thymol observed an inhibition of *Clostridium perfringens* spore germination in ground turkey during chilling. The colony forming units of *Salmonella* and *shigella spp* in ileo-caecal digesta of birds on garlic oil 60ppm, streptomycin sulphate, garlic oil 40ppm and that of birds on control were significantly different (P<0.001) from each other. Ben-Mahdi *et al* (2010) studied the effect of the thyme essential oil in the improvement of growth performance and sanitary status of broiler chickens and observed a significant reduction (P<0.05) of the number of CFU of *Escherichia coli* in the groups supplemented with thyme essential oil compared with the control .

The effects of garlic oil and *streptomycin* sulphate supplementations on nutrients absorptionshowed that the values of apparent digestibility of dry matter from birds on garlic oil 40ppm, garlic oil 60ppm and streptomycin sulphate did not differ but were significantly greater (P<0.01) than

Table 7: Broilers' sex effect of garlic essential oil and *streptomycin sulphate* supplementations on nutrients digestibility

Param-eters	Males	Female	SEM	P value
ADDMa	58.32	62.66	3.540	0.600
ADCP	49.00	53.96	4.694	0.586
ADCF	1.68	3.22	5.124	0.762
ADEE	89.58	90.98	0.838	0.388
CaAR	17.93	20.63	7.042	0.952
PiAR	1.49	5.43	4.820	0.992

Table 8: Treatment x Sex interaction of garlic essential oil and *streptomycin sulphate* supplementations on nutrients digestibility

Parameters	Control		GEO 40ppm		GEO 60ppm		SS 30ppm		SEM	P value
	Male	Female	Male	Female	Male	Female	Male	Female		
*ADDM	58.32^{c}	62.66^{cb}	71.56^{a}	74.37^{a}	72.20^{a}	71.95^{a}	71.03^{a}	69.49^{ba}	3.540	0.032
ADCP	49.00^{c}	53.96^{cb}	63.88^{ba}	67.35^{a}	64.74^{a}	65.09^{a}	61.50^{ba}	60.11^{ba}	4.694	0.046
ADCF	1.68^{b}	3.22^{ba}	13.75^{ba}	20.93^{a}	15.01^{ba}	14.88^{ba}	17.07^{ba}	12.94^{ba}	5.124	0.004
ADEE	89.58^{c}	90.98^{cb}	93.01^{ba}	93.83^{a}	93.10^{ba}	93.10^{ba}	92.17^{cba}	92.05^{cba}	0.838	0.002
CaAR	17.93^{d}	20.63^{dc}	42.05^{ba}	48.82^{a}	45.03^{ba}	40.61^{ba}	34.66^{cba}	30.82^{dcb}	7.042	0.042
PiAR	1.49^{d}	5.43^{dcb}	16.44^{ba}	20.81^{a}	12.90^{cba}	8.40^{dcba}	8.94^{dcba}	5.28^{dcb}	4.820	0.020

a,b,c,d Mean values in the same row with different superscripts are significantly different.
*GEO: Garlic Essential Oil ; SS: Streptomycin Sulphate
*ADDM : Apparent digestibility of dry matter, ADCP : Apparent digestibility of crude protein, ADCF : Apparent digestibility of crude fibre
ADEE : Apparent digestibility of ether extract, Ca AR: Calcium Absorption Rate, Pi AR : Inorganic phosphorus Absorption Rate

that of birds in the control group. This result followed the same pattern in the apparent digestibility of crude fibre, apparent digestibility of fats, except for the calcium and inorganic phosphorus absorption rate. These results tally with the findings of Garcio *et al.,* 2007 who studied the effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers and reported an improvement in apparent ileal digestibility of nutrients in birds on supplemented diets with plant extracts compared with those on the control.

Apparent digestibility of crude protein mean values of birds on garlic oil 40ppm and garlic oil 60ppm did not differ but were significantly higher ($P<0.05$) than the lowest value observed in birds on the control diet whereas that of birds on streptomycin sulphate supplementation was statistically similar to both. The same observation was made for the calcium absorption rate. Loh *et al* (2008) studied the effects of feeding phytogenic substances and phytase on growth performanace and nutrient digestibility of young broilers and reported that birds on supplemented diets had better digestibility of nutrients such as crude protein, phosphorus and calcium compared with the control.

Conclusion

It was concluded that diets fortified with garlic essential oil at minimum 40ppm level of inclusion could efficiently control enteropathogens and improve upon nutrients digestibility while boosting the immune system for a good health and carcass yield of broiler chickens.

References

Adams R P. 2001 Identification of essential oil components by Gas Chromatography/Mass Spectrometry. *Allured Publishing, Carol Stream*, IL.

Alçiçek A, Bozkurt M and Çabuk M. 2004. The effects of a mixture of herbal essential oil, an organic acid or a probiotic on broiler performance. *South African Journal of Animal Science* 34: 217-222.

A.O.A.C 1995. Official Methods of Analysis. 16th edition. *Association of Official Analytical Chemists*, Arlington, Virginia.

Apajalahti J H and Bedford M R.1999 Improve bird performance by feeding its microflora. World Poultry, v.15, pp.20-23.

Apajalahti J H, Kettunen A, Bedford M R *et*

*al.*2001 Percent G+C profiling accurately reveals diet-related differences in the gastrointestinal microbial community of broiler chickens. *Applied Environmental Microbiology*, v.67, p.5656-5667

Apajalahti J H. 2004 Microbial management: a new approach to development in animal nutrition. In: International Congress For The Feed Industry In Southern Africa, 5, 2004, Sun City. Proceedings. Sun City, South Africa: AFMA

Ben-Mahdi M-H, Djellout B, Bouzagh-Belazouz T, Yahiaoui F et Ben-Mahdi N-R., 2010: Intérêt de l'huile essentielle de thym dans l'amélioration des performances zootechniques et sanitaires du poulet de chair. *Livestock Research for Rural Development.* Volume 22, Article #112. Retrieved July 24, 2010, from http://www.lrrd.org/lrrd22/6/benm22112.htm

Cebra J J.1999 Influences of microbiota on intestinal immune system development. *American Journal Clinical Nutrition*, v.69, p.1046-1051

Dorman H J D and Deans S G. 2000. Antimicrobial agent from plants: antimicrobial activity of plant volatile oils. *J. Appl. Microbiol*. 88: 308-316.

Duncan D B. 1955 *Multiple range and F-test. Biometrics* 11:1-42

Ewing W N and Cole D J A 1994 The living gut: an introduction to micro-organisms in nutrition. Context, Dungannon, Ireland.

Frazer C M, Bergeron J A, Mays A and Aiello S E. 1991 Chemotherapeutics. *The Merck Veterinary Manual, 7th edition.* Merck Inc, New Jersey. pp1432 -1435.

Fuller R and Perdigón G. 2003 Gut flora, nutrition, *immunity and health*. Ames: Blackwell Publishing.

García V, Catalá-Gregori P, Hernández F, Megías M D and Madrid J. 2007 Effect of Formic Acid and Plant Extracts on Growth, Nutrient Digestibility, *Intestine Mucosa Morphology, and Meat Yield of Broilers. Journal of Applied Poultry Research.* 16: 555-562

Gill C. 1999. Herbs and plant extracts as growth enhancers. *Feed Int.* 20 (4), 20-23.

Guo F C. 2003 Mushroom and herb polysaccharides as alternatives for antimicrobial growth promoters in poultry. Ph.D dissertation, Wageningen Institute of Animal Sciences, *Department of Animal Nutrition,* P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Guo F C, Williams B A and Kwakkel R P. 2004 Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science,* v.83, p.175-182

Hernandez F, Madrid J, Garcia V, Orengo J and Megias M D. 2004. Influence of two plant extracts on broiler performance, digestibility, and digestive organ size. *Poult. Sci.* 83: 169-174

Hume M E, Clemente-Hernández S and Oviedo-Rondón E O. 2006 Effects of feed additives and mixed Eimeria species infection on intestinal microbial ecology of broilers. *Poultry Science,* v.85, p.2106-211.

Hume M E, Kubena L F and Edrington T S *et al.,* 2003 Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. *Poultry Science* , v.82, n.7, p.1100-1107.

Jamroz D, Wiliczkiewicz A, Wertelecki T, Orda J and Sukorupinska J. 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. British *Poultry. Science.* 46: 485-493.

Johnston A and Booth C. 1983 Plant Pathologist's Pocket Book. 2nd Edn., *Surrey, Commonwealth Agricultural Bureaux,* UK., ISBN: 0851985173.

Juneja V and Friedman M 2007 Carvacrol, cinnamaldehyde, oregano oil, and thymol inhibit Clostridium perfringens spore germination in ground turkey during chilling. Journal of Food Protection ;70[1]:218-222.

Langhout P. 2000. New additives for broiler chickens. *World Poultry-Elsevier* 16[3], 22-27.

Loh T C, Ling H G, Thanh N T, Foo H L, Rajion M A and David S I. 2008. Effects of feeding phytogenic substances and phytase on growth

perfomrnace and nutrient digestibility of young broilers. *Journal of Applied Animal. Research*., 33[2]: 187-192.

Madrid J, Hernandez F, Garcia V, Orengo J, Magias D and Savilla V. 2003. Effect of plant extracts on the ileal apparent digestibility and carcass yield in broilers at level of farm. 14 European Symp. *Poultry Nutrition.* August 10-14, Norway. pp. 187-188.

Nalian A, Oviedo-Rondón E O and Dowd, S. 2009 Effects of essential oil blends on intestinal microbiota of broilers challenged with mixed Eimeria species (In press).

Oviedo-Rondón E O, Hume M E and Hernández C *et al.,* 2006 Intestinal microbial ecology of broilers vaccinated and challenged with mixed Eimeria species, and supplemented with essential oil blends. *Poultry Science,* v.85, p.854-860.

Parker J, Oviedo-Rondón E O and Clementehernández S. 2007 Enzymes as feed additive to aid in responses against Eimeria spp in coccidia vaccinated broilers fed corn-soybean meal diets with different protein levels. *Poultry Science*, v.86, p.643-653.

Radostits O M, Blood D C, and Gay C C.., 1997 Principles of antimicrobial therapy. Veterinary medicine; A text book of the diseases of cattle, sheep , pigs, goats, and horses. 8th edition .W.B.Saunders Company Ltd. 147p

Sinclair J B and Dhingra O D. 1995. Basic Plant Pathology Methods. 2nd Edn., CRC Press, USA., ISBN: 0873716388.

Soxhlet F. 1879 Die gewichtsanalytische Bestimmung des Milchfettes, Polytechnisches J. (Dingler>s), 232, 461

Steel R G D and Torrie J H. 1980 Principles and Procedures of Statistics. A Biometric Approach. Mc Grow Hill book Co.Inc, New York,USA, 2d edition. Pp.191-193

Ultee A, Kets E P W and Smid E J. 1999. Mechanisms of action of carvacrol on the food borne pathogen Bacillus cereus. *Appl. Environ. Microbiol.* 65, 4606-4610.

Zhang K Y, Yan F, Keen C A and Waldroup, P W. 2005.Evaluation of microencapsulated essential oils and organic acids in diets for broiler chickens. Int. *J. Poult. Sci.*4 (9): 612-619

SHORT COMMUNICATION

TWO CASES OF PARATUBERCULOSIS IN UGANDAN CATTLE

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Paratuberculosis or Johne's disease is a chronic granulomatous enteritis of ruminants and wild life species, characterised by chronic diarrhoea, progressive wasting, recumbence and death. It is aused by an acid-fast bacterium called *Mycobacterium* avium subspecies. *paratuberculosis* (Clarke, 1997). Since its discovery in a cow in Germany in 1895, by Johne and Frothingham, the disease has continued to spread into other countries and has acquired new animal hosts (Harris and Barletta, 2001). However, in most African countries, Johne's disease has been considered non-existent. There is scarcely any information about the disease in the continent, even from the World Animal Health Disease Information Database (OIE, 2004). Only Kenya, Rwanda, Cameroon and Lesotho have reported single incidences of the disease in the last 5 years. The only report available on the prevalence of the disease in an African country is from Egypt (Salem et al., 2005).

Introduction of Johne's disease into native herds and cattle populations is through the introduction of an infected animal, which later on contaminates pasture, water and pooled colostrum, thereby infecting susceptible members of the herd, especially calves (Collins,2003; Harris and Barletta, 2001). For almost four decades, Uganda, like other African countries, has been importing cattle of various breeds from Europe, Australia and America, where paratuberculosis has a significant prevalence. However, there has not been any confirmed case of Johne's disease in cattle from Uganda, although lesions suggestive of Paratuberculosis have been observed (Ojok, *Personal communication*). Kirabo (2002) also observed similar lesions during abattoir investigations. We therefore decided to carry out a seroprevalence study of the disease. The cows in this report tested positive to a commercial ELISA (Institut Pourquier, Montpellier, France) during the aforementioned investigation. They were from a ranch in Masindi district located approximately 180 km north-west of Kampala.

The purpose of this paper is to describe and report the occurrence of Johne's disease among ranched cattle in Uganda and stimulate interest in the surveillance of the disease.

Case 1

A Boran cow began to have diarrhoea and experience weight loss. It was treated against trypanosomiasis and helminthiasis which are rampant in the area but without any improvement. During the above-mentioned survey, it reacted positive for the *paratuberculosis*. The cow continued to lose condition until it became recumbent. The management of the ranch decided to offer it for necropsy.

On gross examination, the animal was very emaciated. The most significant finding was the presence of markedly enlarged intestines and mesenteric lymph nodes. The mucosa of the intestine was markedly thickened and corrugated. Histologically, the intestinal sub-mucosa and lamina propria were markedly distended with large numbers of epithelioid cells, macrophages and langhan giant cells. The lymph nodes were oedematous. Numerous langhan *giant* cells, epithelioid cells and *macrophages* had infiltrated both

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the cortex and the medulla. Clumps of acid-fast bacteria were seen in both intestinal tissues and lymph node on Ziehl Nielsen stained sections.

Case 2

Five months after the first case of Johne's disease was recorded in the Boran herd, a Sahiwal cow which had also reacted positive to the ELISA developed chronic diarrhoea and wasting; and continued to deteriorate, but was very alert and had good appetite. It was also sacrificed for postmortem examination.

Grossly. The animal was severely emaciated. On opening the abdominal cavity, the intestine was seen to be generally enlarged, and the lymphatics were markedly dilated. Mesenteric lymph nodes were very prominent, about 3-4 times their normal sizes. The ileocaecal lymphnode in particular measured 5x 6x11cm. The cut surface of lymph nodes and sections of the intestines had yellowish granules, especially the ileoceacal junction associated lymph node. These intestinal lesions involved the entire intestine extending from the torus pylorus to the rectum.



Figure 1. The ileocecal valve (Arrow) and the caecum: Note the marked corrugation, hyperemia and petechiation of the mucosa.
Bar = 2cm

The wall of the intestines was markedly thickened and folded in both transverse and longitudinal patterns (Fig 1). The folds were most prominent in the duodenum, mid ileum, caecum, colon and less pronounced in the jejunum and rectum. The mucosal surface of the intestine was congested, and had petechial hemorrhages and mucoid exudates, most especially in the mid ileum, caecum and colon junction. There was no significant gross lesion in the liver, spleen, lungs, heart and great vessels, kidneys and reproductive tracts.

Histologically, numerous epithelioid cells and macrophages that led to marked widening of the sub mucosa (Fig 2) characterized intestinal lesions. The lamina propria was markedly infiltrated by these cells. Few macrophages were seen in the muscular layer but without any serosal involvement. Application of Ziehl Nielsen stain revealed large numbers of acid-fast bacilli in clumps and singly in all stained intestinal and lymph node sections. Numerous macrophages, *epithelioid* cells, langhan giant cells were seen in the cortex, paracortex and medulla of the lymph nodes, destroying and replacing nearly whole follicles. Some lymph nodes had thickened capsules and widening of the paracortical sinuses. A few necrotic foci were observed in the lymph node. Foci of granulomatous lesions with langhan giant cells were seen in the liver and spleen; however, these foci did not reveal any acid fast organisms when stained with Ziehl Nielsen stain.

A section of the ileum and the associated lymph node were submitted to the National Tuberculosis laboratory, Wandegeya for culture of *M. avium* subspecies. paratuberculosis. The tissues were processed for culture as described by Oloya (2006) and cultured in accordance with the Manual of Diagnostic techniques (OIE, 2008).

Growth of *M.avium* subspecies paratuberculosis was observed from the 13th week as innumerable small, approximately 1mm glistening and transparent colonies on

mycobactin J enriched Herrold's Egg Yolk Medium (HEYM). No growth was seen on HEYM without mycobactin. Smears made from the culture and stained with Ziehl Nielsen stain showed acid-fast organisms. Characteristic mycobactin dependence of the organisms was preserved even after subculture.

The herds from which these cases were identified consist of animals that descended from the original stock of imported cattle, as both Boran and Sahiwal breeds are not native to Uganda. Herd history indicated that animals with similar clinical signs had been previously observed on the ranch. There is indication that the organism can survive within the Ugandan environment and spread to other susceptible hosts. The finding of serologically positive cows in the Ankole, Friesian, Boran and Sahiwal cattle (data not shown) attests to this fact.

Lesions described in this report are consistent with what has been documented in other studies on Johne's disease (Buergelt *et al.,* 1978). However, in the second case described above, the severity and extent of the lesions exceeded what has been described (Clarke,1997). The lesions involved the entire intestine, from the pylorus to the rectum. This has been observed only in experimental cases (Tailor, 1953).

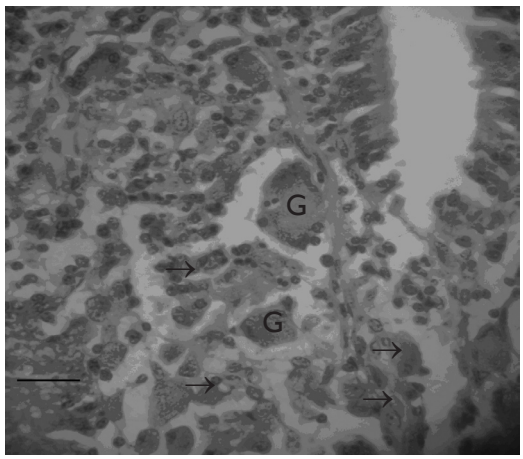


Figure 2. Note the infiltration of mucosa with Giant cells G, Epithelioid cells (arrow-heads). Bar = 100 µm. (Haematoxylin and eosin stain).

In conclusion, Johne's disuse infection is now one of the diseases to be considered in Uganda whenever symptoms such as diarrhoea and wasting are encountered. The new threat by paratuberculosis should be taken seriously and not be allowed to become endemic in the country. There is need to carry out further studies on the epidemiology, pathology and charaterisation of different strains of *M. avium* subspecies paratuberculosis in Uganda.

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References

Buergelt CD, Hall C, McNtee K and Duncan JR, 1978. *Pathological evaluation of paratuberculosis in naturally infected cattle. Veterinary Pathology,* 15: 196-207.

Clarke C J, 1997. The pathology and *pathogenesis* of *paratuberculosis* in ruminants and other species, *Journal of Comparative Pathology*, 116: 217-261.

Collins M T, 2003. Update on *paratuberculosis*: Epidemiology of Johne's disease and biology of *Mycobacterium paratuberculosis*. Irish *Veterinary Journal*, 56(11): 565-574.

Harris N B and Barletta R G, 2001. *Mycobacterium avium subsp*. paratuberculosis in Veterinary medicine. *Clinical Microbiology Reviews,* 14(3):

489-512.

Kirabo A, 2002. Gross and *histopathological* survey of *paratuberculosis* in cattle slaughtered at the Uganda meat industries abattoir. Unpublished special project report, Makerere University, Kampala.

OIE (2004). World animal health information database. Accessed on February 22, 2008, from www.oie.int/wahid-prod/public.php.

OIE (2008). Manual of Diagnostic tests and vaccines for terrestrial animals, 2008. Chapter 2.1.11, *Paratuberculosis*. Accessed on 15th September, 2008, from www.oie.int/eng/normes/mmanual/A_summry.htm

Oloya J, 2006. Epidemiology of tuberculosis in transhumant cattle and characterization of mycobacteria isolated in Karamoja region and Nakasongola district in Uganda. PhD thesis, *National Veterinary Institute*, Oslo, Norway.

Salem M, Zeid A A, Hassan D, El-sayed A and Zshoeck M, 2005. Studies on Johne's disease in Egyptian Cattle. *Journal of Veterinary Medicine* B, 52: 134-137.

Taylor A W, 1953. Experimental infection with varieties of Mycobacterium johnei isolated from sheep. *Journal of Comparative Pathology*, 63: 368-373.

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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
March 2011

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