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1. **EFFECT OF BREAD WASTE AND MORINGA OLEIFERA LEAVES (BWMO) IN THE BASAL DIET OF RABBIT.** *Ayandiran SK, Odeyinka SM and Odedire JA*..... 131
2. **COCOA PLACENTA MEAL: YIELD ESTIMATES, NUTRIENT COMPOSITION AND RESPONSE OF ALBINO RATS TO DIETS CONTAINING GRADED LEVELS WITH OR WITHOUT XZYMETM AN EXOGENOUS ENZYME- PROBIOTIC COMPLEX.** *Boateng M, Okai D B, Amoah K O, Opoku-Asenso A, Aye K A and Frimpong Y O*..... 141
3. **GROWTH AND CARCASS YIELD OF RABBITS IN TWO HOUSING TYPES ADMINISTERED AQUEOUS EXTRACT OF OYSTER MUSHROOM (PLEUROTUS OSTREATUS).** *O. O. Olatubosun, O. M. Sogunle, O. A. Adeyemi, A. M. Bamgbose and T. S. Aremu*..... 151
4. **ESTABLISHMENT OF NATIONAL LIVESTOCK DATABANK FOR GENETIC IMPROVEMENT PROGRAMMES IN ZAMBIA.** *Idowu Kolawole Odubote*..... 161
5. **EFFETS DU PROTOCOLE ANESTHÉSIQUE ACÉPROMAZINE-KÉTAMINE SUR LES PARAMÈTRES VITAUX ET BIOCHIMIQUES LORS DE L'ORCHIDECTOMIE ET LA CAUDECTOMIE DES CHIENS DANS LES CLINIQUES VÉTÉRINAIRES DE YAOUNDÉ (CAMEROUN).** *J. Kouamo, V. Pouenpene Pekariekoue, A. G and Dongmo Kana*..... 169
6. **PREVALENCE OF BOVINE AND OVINE Paratuberculosis IN KERICHO COUNTY AND KONOI SUB-COUNTY, KENYA.** *Joseph A. Omega, Joseph K. N. Kuria and Levi M. Musalia*..... 181
7. **ASSESSMENT AND CONTROL OF TICK INFESTATION IN KALAHARI RED GOATS REARED IN HUMID TROPICS AND THE EFFICACY OF DIFFERENT ACARICIDES.** *Okwelum N, Akinkuotu O A, Shittu O O and Oluwatosin B O*..... 191
8. **EFFECT OF OIL EXTRACTION METHODS AND ENZYME SUPPLEMENTATION ON METABOLISABLE ENERGY VALUES OF SUNFLOWER SEED MEAL FOR GROWING TURKEYS.** *A O Oso, A O Fafolu, E A Adeyeye and A W Oladimeji*..... 197
9. **PRIORITISATION AND CONTROL OPTIONS FOR TRANSBOUNDARY ANIMAL DISEASES (TADS) AND ZOOSES IN THE EAST AFRICAN COMMUNITY (EAC).** *Olaho-Mukani W, Muruiki S, Abdulaziz M A R, Boussini H, Wesonga T W and Baboucarr J*..... 205
10. **THE EFFECT OF WIND SHEAR STRESS IN WATER QUALITY IN NET CAGE FISH FARMING IN A LAKE OPEN WATER COURSE – THE CASE STUDY OF NHAMBAVALE LAGOON, GAZA PROVINCE, MOZAMBIQUE.** *Célia Inês Mubango Hogueane and António Mubango Hogueane*..... 219

EFFECT OF BREAD WASTE AND MORINGA OLEIFERA LEAVES (BWMO) IN THE BASAL DIET OF RABBIT

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Abstract

Sixty mixed breeds of rabbits were allotted into four treatments in a completely randomized design along eight weeks in order to evaluate the effect of bread waste and Moringa oleifera leaf (BWMO) when added to the diet. Treatment (BWMO) at different levels (BWMO -0, BWMO -25, BWMO -50 and BWMO -100%) was added to mashed diet. The chemical composition of the diet was determined as well as the average daily gain of animals, digestibility, blood components and carcass quality. The chemical composition of bread waste consists of 85.0% DM, 11.0% CP, 0.10% CF, 19.4% EE, 1.60% Ash, and 54.0% NFE. The CP of diets containing BWMO was higher ($p < 0.05$) than BWMO-0. The weight gain of the rabbits fed diet BWMO-100 (17.6g/day) was highest ($p < 0.05$) compared to BWMO-50 (16.6g/day), BWMO-25 (12.1g/day) and BWMO-0 (10.8g/day). As the BWMO level increased in the diet, the digestible energy and protein of diets BWMO-100, BWMO-50 and BWMO-25 were higher ($p < 0.05$) than BWMO-0. The blood cholesterol content of rabbits fed BWMO-0 (41.6) was higher ($p < 0.05$) than diets BWMO-25 (33.8), BWMO-50 (37.5) and BWMO-100 (35.9). The total protein and albumin was higher ($p < 0.05$) in rabbits fed diets containing BWMO. The loin, hind limb and fore limb weights of rabbits fed diet BWMO-100 were higher ($p < 0.05$) than those fed the other diets. It could be concluded that BWMO had positive effects on the performances of rabbits.

Keywords: Utilization, bread waste, Moringa oleifera, rabbits

EFFETS DES DÉCHETS DE PAIN ET DES FEUILLES DE MORINGA OLEIFERA (BWMO) DANS LE RÉGIME ALIMENTAIRE DE BASE DU LAPIN

Résumé

Soixante lapins de races mixtes ont été répartis en quatre traitements selon un schéma complètement aléatoire pour une étude réalisée sur une période de huit semaines afin d'évaluer l'effet de l'adjonction de déchets de pain et de feuilles de Moringa oleifera (BWMO : bread waste and Moringa oleifera) au régime sur les lapins sur la performance des lapins. Le traitement (BWMO) à différents niveaux (BWMO -0, BWMO -25, BWMO -50 et BWMO -100%) a été ajouté à un régime sous forme de purée. La composition chimique du régime alimentaire a été déterminée, ainsi que le gain moyen quotidien des animaux, la digestibilité, les composants sanguins et la qualité des carcasses. La composition chimique des déchets de pain se compose de 85,0% de MS, 11,0% de PB, 0,10% de FB, 19,4% d'EE, 1,60% de cendres et 54,0% d'EEA. La PB des régimes contenant des BWMO était supérieure ($p < 0,05$) à celle des régimes BWMO-0. Le gain pondéral des lapins soumis au régime BWMO-100 (17,6 g / jour) était le plus élevé ($p < 0,05$) par rapport aux BWMO-50 (16,6 g / jour), BWMO-25 (12,1 g / jour) et BWMO-0 (10,8 g / jour). Lorsque le niveau de BWMO a augmenté dans le régime, l'énergie et les protéines digestibles des régimes BWMO-100, BWMO-50 et BWMO-25 étaient supérieures ($p < 0,05$) à celles de BWMO-0. Le taux de cholestérol sanguin des lapins recevant BWMO-0 (41,6) était supérieur ($p < 0,05$) à celui des régimes BWMO-25 (33,8), BWMO-50 (37,5) et BWMO-100 (35,9). Les teneurs en protéines et albumine totales était plus élevée ($p < 0,05$) chez les lapins soumis aux régimes contenant des BWMO. Les poids de la longe, des membres postérieurs et des membres antérieurs des lapins nourris au régime BWMO-100 étaient plus élevés ($p < 0,05$) que ceux des lapins soumis aux autres régimes. On pourrait en conclure que l'adjonction de BWMO a eu des effets positifs sur les performances des lapins.

Mots-clés : Utilisation, déchets de pain, Moringa oleifera, lapins

Introduction

Rabbit production is one way of meeting the animal protein requirement of the Nigeria populace (Iyeghe-Erakpotobor *et al.*, 2012). Rabbits are characterised by their high prolificacy, a good mothering ability, an easy management strategy, the ability to valorise waste and other unconventional feed sources for maximum meat gain (Bassey *et al.*, 2008) including the ability to thrive well on forage (Fielding, 1991) with little concentrate. Moreover, rabbit meat is known to contain high a quality and quantity of protein, less fat with a higher proportion of polyunsaturated linoleic and linolenic fatty acids (Njidda and Isidahomen, 2011). Based on these attributes of rabbits, researchers have focused more interest on improving performance by feeding rabbits with agro-industrial wastes and forages that are cheap and available resources throughout the year. The most common unconventional feedstuffs (agro-industrial wastes) are cassava peel, brewer's dried grain, pineapple waste, flour dust, biscuit waste, bread waste, noodle waste, cocoa pod meal and shrimp waste. Bread waste, a by-product obtained from the bakery industry, is rich in vitamin and energy but low in fibre content (Al-Tulaihan *et al.*, 2004). In Nigeria, *Moringa oleifera*, commonly called the horse radish tree, is an inexpensive forage protein source for livestock feeding (Odeyinka *et al.*, 2008). To the best of our knowledge, there are no studies reported on the utilization of bread waste and *Moringa oleifera* leaves as supplemental feed sources in the diet of rabbits especially during the dry season when native forages are scarce. Therefore, this study was carried out to evaluate the performance, blood components and carcass qualities of rabbits fed on a mixture of bread waste and *Moringa oleifera* leaves (BWMO) when added, at different levels, to basal diet.

Materials and Methods

Animals and experimental design

Sixty weaned rabbits of mixed breeds aged between 5 and 6 weeks were used in

the adaptation (7-days) and study (8 weeks) periods. The rabbits were housed in individual hutches and randomly allocated into four treatment groups (15 rabbits per treatment). Feed and water were served daily ad libitum.

Experimental diets

Bread wastes were collected from a bakery, oven dried at 60°C for 24 hours, packaged in high-density polythene bags and stored for subsequent use. Fresh *Moringa oleifera* leaves were harvested from Obafemi Awolowo University Teaching and Research farm, Ile-Ife, Nigeria and air dried on a concrete floor for 4 days. Four diets were compounded with the inclusion of bread waste and *Moringa oleifera* leaves (BWMO) at 0, 25, 50 and 100% levels (BWMO-0, BWMO-25, BWMO-50 and BWMO-100) as shown in Table 1. The experimental diets were fed to rabbits at 4% of their body weight.

Digestibility study

Animals were subjected to a 7-day digestion trial. The total excreted faeces were collected daily, oven dried, bulked, thoroughly mixed and ground through a 2mm hammer screen for chemical analysis. The digestibility values for dry matter (DM), crude protein (CP), ether extract (EE), and crude fibre (CF) were calculated as nutrient intake minus nutrient excreted divided by nutrient intake multiplied by one hundred. Urine samples collected were stored in a refrigerator at 4°C for chemical analysis. The crude protein content of experimental diets was divided by a factor of 6.25 to calculate the nitrogen intake of rabbits. The faecal and urinary nitrogen contents were determined using the method described by AOAC (2000)

$$\text{Apparent Digestibility} = \frac{\text{Nutrient in feed} - \text{Nutrient in faeces}}{\text{Nutrient in feed}} \times 100$$

$$\text{Nitrogen output} = \text{faecal nitrogen} + \text{urinary nitrogen}$$

$$\text{Nitrogen balance} = \text{Nitrogen intake} - \text{Nitrogen output}$$

$$\text{Nitrogen (\%)} = \frac{\text{Nitrogen balance} \times 100}{\text{Nitrogen intake}}$$

Chemical analysis

Ash, crude protein (CP) and ether extract (EE) and crude fibre (CF) were determined following the methods of the AOAC (2000)

Blood sample collection and analysis

At the end of the feeding period, animals were starved of feed for 24 hours before collecting blood samples. Samples were collected per replicate from each rabbit from the external ear vein using a sterilized disposable syringe and needle between 6.30 am and 7.30 am for haematological and biochemical analysis. An initial 1.0 ml sample of blood was collected into labelled sterile universal bottles containing Ethylene- Diamine-Tetra-Acetic acid (EDTA) as anticoagulant. Another 1.0 ml of blood was collected into labelled sterile bottles without anticoagulant in order to determine the biochemical components. The albumin concentration was determined by the Bromocresol Green (BCG) method (Peters *et al.*, 1982). The globulin (Gb) concentration was computed as the difference between the total protein and albumin concentrations. Cholesterol was determined as described by Coles (1986). The red blood cell (RBC) counts, total white blood cell (WBC) counts, haemoglobin (Hb) concentration and packed cell volume (PCV) parameters were determined following standard procedures described by Device and Lewis (1991).

Determination of animal performance and carcass quality

Animals were weighed before the commencement of the experiment and subsequently at weekly intervals throughout the experimental period wherein the initial weight, final weight and average daily weight gains were calculated.

$$\text{Feed intake} = \text{Feed offered} - \text{Left over}$$

$$\text{Feed conversion ratio} = \frac{\text{Daily feed intake}}{\text{Daily weight gain}}$$

At the end of the experimental period, eight rabbits per treatment were randomly selected, starved of feed for 24 hours, weighed and humanely slaughtered. The jugular vein was cut to allow proper bleeding. Thereafter, the skins, heads, loins, fore limbs, hind limbs, kidneys, kidney fat, intestines, livers, lungs and hearts were removed and weighed (Odeyinka *et al.*, 2008).

Data analysis

Statistical differences among the experimental diets in all the studied parameters were evaluated by one-way analysis of variance (SAS, 2008). The statistical significance of the differences between means was assessed using the Duncan test

Table 1: Gross composition of the experimental diets

Ingredient (%)	BWMO-0	BWMO-25	BWMO-50	BWMO-100
Corn bran	40.00	30.00	20.00	-
Brewer's dried grain	40.00	30.00	20.00	-
Bread waste	-	10.00	20.00	40.00
Moringa oleifera	-	10.00	20.00	40.00
Groundnut cake	17.00	17.00	17.00	17.00
Bone meal	2.00	2.00	2.00	2.00
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Vitamin premix	0.50	0.50	0.50	0.50

¹Premix provided per kg diet: vitamin A, 12,000 IU; vitamin D3, 1,000 IU; vitamin E acetate, 50 mg; vitamin K3, 2 mg; biotin, 0.1 mg; Fe, 100 mg; Cu, 20 mg; Mn, 50 mg; Co, 2 mg; I, 1 mg; Zn, 100 mg; Se, 0.1 mg; Robenidine, 66 mg.

Results

The percentage chemical compositions of bread waste and experimental diets are presented in Table 2. Dry matter contents (DM) varied between 85% (bread waste) and 96 % (BWMO-0). The CP content of BWMO diets increased with increasing inclusion levels but these had much lower EE content compared to bread waste. Data on some performance characteristics are presented in Table 3. There was no significant difference ($p>0.05$) in the mean daily feed intakes of the rabbits fed the experimental diets. Rabbits fed diets BWMO-50 and BWMO-100 had significantly higher ($p<0.05$) daily weight gains compared to BWMO-25 and BWMO-0. There was significance difference ($P<0.05$) in the feed conversion ratio throughout the experimental diets.

There was significant difference ($P<0.05$) among the means of the percentage digestibility coefficient of the rabbits fed bread waste and Moringa oleifera meals (Table 4). The percentage dry matter digestibility decreased as the inclusion of BWMO increased in the

diets. The digestible energy and crude protein of diets BWMO-100, BWMO-50 and BWMO-25 were significantly higher ($p<0.05$) than that of BWMO. There was no significant difference ($p>0.05$) in the digestible ether extract among the experimental diets. The BWMO-0 diet had significantly higher ($p<0.05$) digestible ash followed by BWMO-25, BWMO-50 and BWMO-100. There was significant difference ($p<0.05$) among the means of nitrogen utilization of rabbits fed the experimental diets (Table 5). The nitrogen intake of diets BWMO-0 and BWMO-25 were significantly lower ($p<0.05$) than that of diets BWMO100 and BWMO-50. The nitrogen retention percentage was significantly higher ($p<0.05$) in diets BWMO-100, BWMO-50, BWMO-25 than BWMO-0.

There were no significant differences ($p>0.05$) among the means of the packed cell volume and the white blood cell counts of the animals while the red blood cell counts were significantly higher ($P<0.05$) in diets containing inclusions of BWMO. Furthermore, the serum metabolites were also significantly higher ($P<0.05$) in diets containing BWMO

Table 2: Percentage chemical composition of bread waste and experimental diets

Parameters (%)	Bread waste	BWMO-0	BWMO-25	BWMO-50	BWMO-100
Dry matter	85.00	96.00	92.00	86.00	88.00
Crude protein	11.00	15.20	17.50	19.00	19.20
Crude fibre	10.10	17.00	16.00	12.00	11.00
Ether extract	19.40	5.50	6.50	6.90	6.80
Ash	1.60	10.00	9.20	8.50	7.50
NFE	54.00	38.50	37.70	37.20	36.20

NFE: nitrogen free extract.

Table 3: Performance characteristics of rabbits supplemented with bread waste and Moringa oleifera leaves

Parameters	BWMO-0	BWMO-25	BWMO-50	BWMO-100	SEM	Prob.
Feed intake (g/day)	43.10	45.30	43.60	46.40	0.62	<0.0001
Initial weight (g)	53.50	54.00	53.40	54.10	0.19	<0.0001
Final weight (g)	118 ^d	126 ^c	153 ^b	160 ^a	5.33	<0.0001
Weight gain (g/day)	10.80 ^d	12.10 ^c	16.60 ^b	17.60 ^a	0.88	<0.0001
Feed conversion ratio	3.87 ^a	3.74 ^a	2.56 ^b	2.63 ^b	0.18	<0.0001

^{abc} Means with different letters on the same row differ ($P<0.01$)

Table 4: Apparent digestibility (%) of the experimental diets.

Parameters (%)	BWMO-0	BWMO-25	BWMO-50	BWMO-100	SEM	Prob.
DDM	82.1 ^a	76.4 ^{ab}	77.4 ^b	71.1 ^b	1.84	0.04
DE	66.0 ^c	74.0 ^b	75.4 ^b	79.3 ^a	1.70	0.03
DCP	53.4 ^c	57.8 ^b	61.5 ^a	63.6 ^a	1.30	0.05
DCF	69.4 ^b	58.5 ^c	54.9 ^c	71.8 ^a	1.60	0.01
DEE	61.9	60.3	60.2	58.5	1.50	0.21
DNFE	41.0	39.5	37.8	38.1	0.92	0.50

^{abc} Means with different letters on the same row differ ($P<0.05$). DDM: Digestible dry matter, DE: Digestible energy, DCP: Digestible crude protein, DCF: Digestible crude fibre, DEE: Digestible ether extract, DNFE: Digestible nitrogen free extract.

Table 5: Nitrogen utilization of bread waste and Moringa oleifera leaves supplemented to rabbit's feed

Parameters (%)	BWMO-0	BWMO-25	BWMO-50	BWMO-100	SEM	Prob.
Nitrogen Intake	2.40 ^b	2.70 ^b	3.03 ^{ab}	3.10 ^a	0.37	0.03
Feecal Nitrogen	0.43 ^{ab}	0.39 ^b	0.54 ^a	0.50 ^{ab}	0.02	0.04
Urinary Nitrogen	0.65 ^a	0.47 ^b	0.38 ^c	0.33 ^d	0.05	0.02
Nitrogen Balance	1.42 ^c	1.85 ^c	2.12 ^b	2.43 ^a	0.11	0.03
Nitrogen Retention (%)	57.9 ^b	68.3 ^{ab}	69.9 ^{ab}	78.6 ^a	2.54	0.01
DNFE	41.0	39.5	37.8	38.1	0.92	0.50

^{abc} Means with different letters on the same row differ ($P<0.05$)

Table 6: The blood components of rabbit

Parameters (%)	BWMO-0	BWMO-25	BWMO-50	BWMO-100	SEM	Prob.
PCV (%)	32.2	33.8	34.0	34.8	1.44	0.03
RBC (10^6)	2.84 ^a	4.98 ^a	5.00 ^a	4.93 ^a	0.39	0.04
WBC (10^3)	4.95	4.70	4.89	4.45	0.06	0.31
Cholesterol (mg/dl)	41.6 ^a	33.8 ^b	37.5 ^b	35.9 ^b	1.08	0.05
Total protein (mg/dl)	5.40 ^c	6.43 ^b	6.99 ^{ab}	7.70 ^a	0.33	0.03
Albumin (mg/dl)	3.30 ^b	3.98 ^{ab}	4.15 ^a	4.69 ^a	0.20	0.05
Globulin (mg/dl)	2.10 ^b	2.50 ^a	2.84 ^a	2.87 ^a	0.13	0.02

^{abc} Means with different letters on the same row differ ($P<0.05$). PCV: Packed cell volume RBC: Red blood cell WBC: White blood cell

levels than in the diet with BWMO-0. The blood cholesterol content of rabbits fed diets BWMO-0 was significantly higher ($P<0.05$) than diets BWMO-25, BWMO-50 and BWMO-100. There were no significant differences ($p>0.05$) among the means of the slaughter weights of the rabbits fed experimental diets (Table 7). There was significant difference ($p<0.05$) among the means of the carcass weights with BWMO-100 being the highest followed by BWMO-50, BWMO-25 then BWMO-0. The dressing

percentage and loin weight of rabbits fed diets BWMO-100, BWMO-50 and BWMO-25 were significantly higher ($p<0.05$) than rabbits fed BWMO-0 diet. Likewise, the hind and fore limb weights were significantly higher ($P<0.05$) in rabbits fed diets containing BWMO compared to the BWMO-0 diet. There were no significant difference ($p>0.05$) in the weights of all the visceral organs such as liver, kidney, heart, lungs and spleen across the treatments.

Table 7: The carcass characteristics of rabbits

Parameters (%)	BWMO-0	BWMO-25	BWMO-50	BWMO-100	SEM	Prob.
Slaughter weight	103	105	108	112	32.1	0.64
Carcass weight	816 ^b	883 ^{ab}	825 ^b	988 ^a	27.60	0.11
Loin	228 ^b	325 ^a	237 ^b	312 ^a	17.30	0.05
Head	100	115	113	125	6.210	0.73
Fore limb	25.0 ^b	32.5 ^{ab}	37.5 ^{ab}	55.0 ^a	4.53	0.07
Hind limb	50.5 ^c	52.0 ^c	75.0 ^b	100 ^a	7.64	0.04
Liver	38.1	40.9	39.8	41.8	4.36	0.32
Kidney	9.09	10.6	9.29	9.53	0.79	0.82
Heart	2.79	3.43	2.84	3.07	0.16	0.48
Lungs	4.92 ^b	5.37 ^b	6.81 ^a	6.73 ^a	0.43	0.38

^{abc} Means with different letters on the same row differ ($P < 0.05$)

Discussion

The DM of bread waste in this study was lower than those reported by (Omole *et al.*, 2011) who recorded 98.8% DM. Likewise, CP contents varied between 11% (bread waste) and 19.2% (BWMO-100) higher than the 11.96% reported by Malomo *et al.*, (2011) on composite bread made from breadfruit flour, breadnut flour. Moreover, CP contents of the experimental diets (15.2-19.2%) were higher than those reported by Mmereole *et al.*, (2011) who recorded levels between 12.1% and 16.0%. The levels of CF revealed a linear decrease with increasing BWMO in the diet with the bread waste containing the lowest levels. Moreover the values contained in the experimental diets in this study were fairly higher (11-17%) than those obtained by Al-Shami and Mohammed (2009) for diets containing bread waste crumbs (9.6 - 14.60%). It could therefore be inferred from this study that the inclusion of BWMO in the diet of rabbits provided the nutrients required for maintenance and production functions especially during the dry season.

The daily feed intake values were considered lower than those recorded by Federick (2010) for Moringa based diet (60.1 - 63.4 g/day). Similar results were reported earlier by Mufwa *et al.*, (2011) for rabbits fed brewers dried grains. However, there were slight increases in the numeric values of diets

containing inclusion of BWMO than the other diets. This could be associated with increasing levels of protein in the BWMO diets. The daily weight gain range recorded in this study for diets containing BWMO was higher compared to 6.78 – 8.64 g/day (Odeyinka *et al.*, 2008) for rabbits fed Moringa oleifera as replacement for *Centrosema pubescens*. The higher weight gains in the rabbits fed the BWMO inclusive diets may, therefore, be partly due to a better protein quality, possibly arising from a higher methionine and lysine supply (Booth and Wickens, 1998). The values obtained in this study were lower than the 5.11 – 7.66 g/day reported by Mmereole *et al.*, (2011) for rabbits fed protein diets supplemented with Tridax procumbens and the 2.63- 4.00 g/day reported by earlier researchers in the tropics (Okorie, 2003). This observation could be attributed to enhanced feed intake and the better utilization of diets. Hence increase in weight gain of rabbits fed BWMO lead to improvement in the feed conversion ratio. The digestible dry matter was higher than the 65.02% - 78.40% reported by Odeyinka *et al.*, (2008) for rabbits fed Moringa oleifera diet. The apparent digestibility of CP was higher than 26.28% - 62.48% reported for rabbits in the tropics (Iyeghe-Erakpotobor *et al.*, 2006). The fact that digestible crude protein contents and digestible energy increased as the level of bread waste and Moringa leaf increased in the diets in this study, agreed with the

study of Fahey *et al.*, (2001) that Moringa is an outstanding source of highly digestible nutrients. All animals fed on any of the experimental diets had positive nitrogen balance values. The nitrogen balance in this study were higher than the 1.62 – 1.64 reported by Adeniji and Lawal (2012) for rabbits fed Moringa based diets. The percentage nitrogen retention values for animals fed diets containing bread waste and *M. oleifera* leaf were relatively similar to the 70.31% – 70.56% reported earlier (Adeniji and Lawal, 2012).

The packed cell volume in this study was lower than 43.30% – 46.77% (Federick, 2010) but conformed to (32.00% – 35.00 %) observed by Odetola *et al.*, (2012) for rabbits fed Moringa oleifera leaves as replacement for soybean meal. The rabbits fed diets containing BWMO had higher red blood cell counts which affirmed the claim of Brown *et al.*, (2000) who reported that increased red blood cell count values are associated with high quality dietary protein and with disease free animals. Also the white blood cell count conformed to 4.34 – 5.88 x 10⁶/mm³ observed by Dairo and Egbeyemi (2012) in rabbits fed mixture of cassava peels and layer's manure. The blood cholesterol levels conformed to 35.02 – 40.70 mg/dl obtained by Federick (2010) for rabbits fed *M. oleifera* leaf and also fell within the normal physiological range of 35 – 60 mg/dl reported by Jenkins (1993). The blood cholesterol levels reduced as the level of inclusion of BWMO increased in the experimental diets. This observation agrees with the results of research by Ghasi *et al.*, (1999) where they reported that juice extracted from Moringa leaves was found to be a potent hypocholesterolemic agent. The values for total protein, albumin and globulin obtained in this study were found to be within the normal physiological range for rabbits; total protein (5.4 – 7.5 g/dl), albumin (2.5 – 4.5 g/dl) and globulin (1.9 – 3.5 g/dl) (Hillyer, 1994). Hence, BWMO in the diet of rabbits had no adverse effect on the blood components. The carcass weight values here were comparable to 764.79 – 878.00 g reported by Ojebiyi *et al.*, (2013) but higher than 354.31 – 738.40 g (Adekojo *et al.*, 2014) for rabbits fed processed

Leuceana leucocephala leaves. The loin and head weights in this study were higher than the values reported by Ngoshe *et al.*, (2013). Generally, the carcass quality parameters of rabbits fed diets containing BWMO were superior compared to rabbits fed the other diet. This agreed with the observations of Federick (2010) for rabbits fed diets with inclusions of Moringa leaves. The weight of the internal organs across the treatments in this study were better than values of rabbits fed cowpea and soybean hull based diets (Orji, 2009). Ahamefule *et al.*, (2006) reported that the weights of some internal organs like kidney and liver may be used in animal feeding experiments as evidence of toxicity. The values obtained for the internal organs in this study were within the normal range, hence indicating that there were no abnormalities or pathological lesions in these organs, thus confirming no toxicity in the treatment diets.

Conclusion

It could be concluded that BWMO had positive effects on the feed intake, weight gain, nutrient digestibility, blood components and carcass qualities of rabbits. Therefore further studies into the utilization of bread wastes as supplemental feed source for rabbits especially in the dry season is required. .

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COCOA PLACENTA MEAL:YIELD ESTIMATES,NUTRIENT COMPOSITION AND RESPONSE OF ALBINO RATS TO DIETS CONTAINING GRADED LEVELS WITH OR WITHOUT XZYMETM AN EXOGENOUS ENZYME- PROBIOTIC COMPLEX

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Abstract

Two separate 4-week feeding trials were conducted to determine the effects of partially replacing maize with varying levels of cocoa placenta meal (CPM) on the growth performance, economies of production and carcass traits of monogastric farm animals using albino rats as a model. Before the feeding trials, 100 cocoa pods were collected and used to estimate the proportion of the cocoa fruit which constitutes the placenta. The values obtained served as a basis for estimating the country's potential CPM yield. The proximate composition of the CPM was determined before the individual experimental diets used in the 2 studies were formulated and compounded. Each feeding trial involved 20 rats (12 males and 8 females) with a mean initial weight of 75.7g and 58.1g for the first and second feeding trials respectively. Four iso-nitrogenous dietary treatments were used in both experiments. Treatment diets for the first trial were designated: STIT1 – i.e. basal diet containing no CPM (Control), and STIT2, STIT3 and STIT4 containing 5, 10 and 15% CPM respectively. Dietary treatments used in the second trial were labeled: ST2T1 – i.e. basal diet containing neither CPM nor XZYMETM, and ST2T2, ST2T3 and ST2T4, containing 5, 10 and 15% CPM respectively. The dietary treatments in the second study containing CPM were supplemented with 250mg/kg feed of XZYMETM, an exogenous enzyme- probiotic complex. The experiments were laid out in randomized complete block designs and blockings were appended based on sexes and weights of the rats. Each replicate consisted of a rat and there were 5 rats in every treatment. After both trials, rats were euthanized for carcass analyses. An average estimate of 98,403.6 MT of cocoa placenta was found to be generated annually in Ghana from the year 2000 to 2010. Proximate analysis of CPM samples indicated 14.88, 3.37, 3.0, 2.38 and 63.17% crude protein, ash, fat, crude fibre and nitrogen-free extracts respectively on as-fed basis. There were no significant ($P > 0.05$) differences in feed intake and live weight changes in both trials. Replacing maize with CPM-containing diets reduced feed cost by 5, 10 and 14% in treatments STIT2, STIT3, STIT4 respectively (trail 1). In trail 2, after XZYMETM had been added to CPM-containing diets, diets ST2T2, ST2T3, ST2T4 were 4, 9 and 13% respectively cheaper than the Control (ST2T1). Feeding CPM-based diets had no effects on vital organs with the exception of heart which was significantly ($P < 0.05$) smaller in diets containing 15% CPM without XZYMETM. The study showed that Ghana produces a substantial amount of cocoa placenta which can be used in livestock feeding especially in cocoa growing areas. Again, feeding diets containing up to 15% CPM with or without XZYMETM had no adverse effects on the health, growth performance and carcass characteristics of the experimental rats.

Keywords: cocoa placenta, XZYMETM, growth, albino rats, carcass traits

FARINE DE PLACENTA DE CACAO : ESTIMATIONS DE RENDEMENT, COMPOSITION EN ÉLÉMENTS NUTRITIFS ET RÉACTION DES RATS ALBINS AUX RÉGIMES CONTENANT DES NIVEAUX GRADUELS AVEC OU SANS XZYMETM, UN COMPLEXE EXOGÈNE ENZYME-PROBIOTIQUE

Résumé

Deux essais d'alimentation distincts d'une durée de quatre semaines ont été menés pour déterminer les effets du remplacement partiel du maïs par des taux variables de farine de placenta de cacao (CPM) sur les performances de croissance, les économies de production et les caractéristiques de carcasse d'animaux de ferme monogastriques en utilisant des rats albins comme modèle. Avant les essais d'alimentation, 100 cosses de cacao ont été collectées et utilisées pour l'estimation de la proportion de

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fruit de cacao constituant le placenta. Les valeurs obtenues ont servi de base à l'estimation du rendement potentiel de la CPM du pays. La composition immédiate de la CPM a été déterminée avant la formulation des régimes expérimentaux individuels utilisés dans les 2 études. Chaque essai d'alimentation comprenait 20 rats (12 mâles et 8 femelles) avec un poids initial moyen de 75,7 g et 58,1 g, respectivement pour le premier et le deuxième essais d'alimentation. Quatre traitements diététiques iso-azotés ont été utilisés dans les deux expériences. Les régimes de traitement pour le premier essai ont été désignés de la manière suivante : STIT1 – c.-à-d. un régime de base ne contenant pas de CPM (témoin), et STIT2, STIT3 and STIT4 contenant respectivement 5, 10 et 15% de CPM. Les traitements diététiques utilisés dans le deuxième essai étaient : ST2T1 - c.-à-d. un régime de base ne contenant ni CPM ni XZYMETM, et ST2T2, ST2T3 et ST2T4, contenant respectivement 5, 10 et 15% de CPM. Les traitements diététiques de la deuxième étude contenant de la CPM ont été complétés avec 250 mg / kg de XZYMETM, un complexe exogène enzyme-probiotique. Les expériences ont été présentées sous forme de blocs complets randomisés, et des blocages ont été ajoutés en fonction du sexe et du poids des rats. Chaque répétition consistait en un rat, et il y avait 5 rats dans chaque traitement. Après les deux essais, les rats ont été euthanasiés pour des analyses de carcasses. Au Ghana, il a été estimé que la production annuelle de placenta de cacao s'élevait en moyenne à 98 403,6 tonnes métriques de 2000 à 2010. L'analyse immédiate des échantillons de CPM a révélé des taux de 14,88, 3,37, 3,0, 2,38 et 63,17% respectivement pour les protéines brutes, les cendres, la graisse, les fibres brutes et les extraits exempts d'azote, exprimés sur produit brut. On n'a pas relevé de différences significatives ($P > 0,05$) au niveau de la consommation d'aliments et des changements de poids vif dans les deux essais. Le remplacement du maïs par des régimes contenant de la CPM a permis de réduire les coûts d'alimentation de 5, 10 et 14% respectivement pour les traitements STIT2, STIT3, STIT4 (piste 1). En piste 2, après l'adjonction du complexe XZYMETM aux régimes contenant de la CPM, les régimes ST2T2, ST2T3, ST2T4 étaient respectivement 4, 9 et 13% moins chers que le groupe témoin (ST2T1). Les régimes alimentaires à base de CPM n'ont eu aucun effet sur les organes vitaux, à l'exception du cœur qui était significativement plus petit ($p < 0,05$) dans les régimes contenant 15% de CPM sans XZYMETM. L'étude a montré que le Ghana produit une quantité substantielle de placenta de cacao qui peut être utilisé pour l'alimentation des animaux, en particulier dans les zones de production de cacao. Encore une fois, les régimes alimentaires contenant jusqu'à 15% de CPM avec ou sans XZYME TM n'ont eu aucun effet indésirable sur la santé, les performances de croissance et les caractéristiques des carcasses des rats expérimentaux.

Mots-clés : placenta de cacao, XZYMETM, croissance, rats albinos, caractéristique de carcasse

Introduction

Animal production in developing countries like Ghana is hindered by the high cost and inadequate supply of feed. According to the Food and Agriculture Organization (FAO, 2014), feed costs alone represents over 70% of the total cost of production of pigs, with the energy component being the greatest proportion and forming between 45 to 60% of the diet of monogastric livestock. This high cost has been attributed to the increased demand for certain feed resources caused by the competition between humans and animals for the same feed/foodstuffs. Livestock are therefore poorly fed (Katona and Katona-Apte, 2008) and this has resulted in poor health and growth, increase in livestock mortality and a drastic reduction in the profit margins of

livestock farmers.

Although approaches such as breeding and improved management systems may be helpful in addressing this issue, one viable solution is the use of by-products and other secondary products generated after the processing of human food and other industrial products (Devendra and Leng, 2011). In Ghana, some work has been done on by-products such as pawpaw by-products (Boateng *et al.*, 2014), rice by-products (Atuahene *et al.*, 2000), brewers' spent grains (Amoah *et al.*, 2013), yam by-products (Donkoh *et al.*, 2013) and the African locust bean (*Parkia biglobosa*) by-products (Pelig-Ba, 2009).

Ghana is second to Cote d'Ivoire in the production and exportation of cocoa beans. Besides cocoa beans, cocoa yields by-products like the husk and placenta. Some of

these by-products are disposed of haphazardly and become wastes and pollutants to the environment. In Ghana, the husk of cocoa is burnt and used as fertilizer or potash in the making of soap. The placenta in most cases is fermented along with the beans and gradually removed during the process of drying. The authors of this current study however, observed that some pig farmers in some cocoa growing areas in Ghana gather and feed dried cocoa placenta to their pigs. It is in the light of this that this study was carried out to determine the potential annual yield and nutrient composition of cocoa placenta meal (CPM), and also evaluate the effect of feeding diets containing varying levels of CPM with or without XZYMETM (an exogenous enzymes-probiotic complex) on the growth, economics of production and carcass traits of monogastric livestock using albino rats as a model.

Materials and Methods

Location and Duration of Experiment

The study consisted of an estimation of the potential annual yield and proximate analysis of the CPM, and two (2) feeding trials to estimate the effects of varying levels of CPM with or without XZYMETM (a probiotic and exogenous enzyme complex) (P & C Animal Health LLP, India) on the growth performance, economics of production and carcass composition of monogastric farm animals using albino rats as a model. In the first trial, rats were fed diets containing varying levels of CPM whilst in the subsequent trial, 250mg of XZYMETM was added to every kg of the CPM-containing diets.

The proximate analysis of the CPM and feeding trial were carried out at the Nutrition Laboratory and the Livestock Section respectively of the Department of Animal Science, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Each of the feeding trials spanned a period of four weeks (28 days).

Source and processing of feed ingredients

Cocoa placentas were obtained from

the Plantations Section of the Department of Crops and Soil Sciences, KNUST, Kumasi, Ghana. They were air-dried for four (4) days. The other feed ingredients were bought from the open market in Kumasi whilst the XZYMETM was obtained from Gokal Ltd, Kumasi, Ghana. Before the various experimental diets were compounded, all ingredients were milled to pass through a 2mm sieve with a laboratory hammer mill (Christy and Norris Limited, Suffolk, UK).

Proximate analysis of CPM

Proximate analysis was carried out on the CPM using standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2002). Metabolizable energy (ME) of the CPM was however calculated using the equation:

Metabolizable energy (kcal kg⁻¹) = 37 × % crude protein (CP) + 81.8 × % ether extract (EE) + 35 × % Nitrogen Free Extracts (NFE) as proposed by Pausenga (1985). Nutrient composition of all other ingredients were obtained from the NRC (1998).

Experimental animals, design and diets

Each of the two feeding trials had twenty (20) albino rats (12 males and 8 females). The rats used for the first feeding trial had an average initial weight of 75.7g whilst those used for the latter trial were of an average weight of 58.1g. All rats were obtained from the Animal House of the Faculty of Pharmacy and Pharmaceutical Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Each feeding trial was laid out as a randomized complete block design and blocking was based on the weights and sexes of the rats. Four iso-nitrogenous (17.2% CP) dietary treatments were used in both trials (Table 1). The dietary treatments used in Trial 1 were STIT1 – i.e. basal diet containing no CPM (Control), and STIT2, STIT3 and STIT4 containing 5, 10 and 15% CPM respectively. Dietary treatments used in Trial 2 were ST2T1 (Control), a basal diet containing neither CPM nor XZYMETM, and ST2T2, ST2T3,

Table 1: Percentage composition of the experimental diets (% as-fed basis)

INGREDIENTS	DIETARY TREATMENT ^β			
	ST ₁ /ST ₂ T ₁ (0% CPM)	ST ₁ /ST ₂ T ₂ (5% CPM)	ST ₁ /ST ₂ T ₃ (10% CPM)	ST ₁ /ST ₂ T ₄ (15% CPM)
Cocoa placenta meal [§]	0	5	10	15
Maize	60	55	50	45
Soya bean meal	11	10	9	8
Wheat bran	23	24	25	26
Fish meal	5	5	5	5
Dicalcium phosphate	0.25	0.25	0.25	0.25
Vit-trace min. premix ¹	0.25	0.25	0.25	0.25
Common salt	0.5	0.5	0.5	0.5
Total	100	100	100	100
Calculated Nutrient composition				
Crude protein (%)	17.2	17.2	17.2	17.2
Crude fiber (%)	4.75	4.76	4.78	4.79
ME (kcal/kg)	3093.05	3063.35	3033.64	3003.94

^βST₁/ST₂ represent Trials 1 and 2 respectively.

[§]XZYME was added at the rate of 250mg per kg of diet for Trial 2 in CPM-containing diets. Every kg of XZYMETM contained: Lactic acid Bacillus (30 × 10⁹ CFU), Saccharomyces cerevisiae (10 × 10¹⁰ CFU), Amylase (29,000 IU), Betaglucanase (405,000 IU), Phytase (44,500 IU), Lipase (31,000 IU), Protease (740,000 IU), Cellulase (5,500 IU), Pectinase (101,000 IU) and Hemicellulase (25,000 IU). Vitamin-trace mineral premix provided the following per kg of diet: Vitamin A (8000 I.U); Vitamin D3 (150I.U); Vitamin E (2.5mg); Vitamin K (1mg); Vitamin B2 (2mg); Vitamin B12 (5 × 10⁻³mg); Folic Acid (0.5mg); Nicotinic Acid (8mg); Calcium Panthotenate (2mg); Choline Chloruro (50mg), Trace Elements: Mg (50mg); Zn (40mg); Co (0.1mg); Cu (4.5mg); Se (0.1mg). Antioxidants: Butylated Hydroxytoluene (10mg). Carrier: Calcium carbonate q.s.p.

ST2T4 which contained 5, 10 and 15% CPM respectively. In Trial 2, 250 mg of XZYMETM per kg feed was added to all CPM-containing diets. For both experiments, each treatment was replicated five (5) times and there was one rat in each replicate.

Management of rats

The rats were housed individually in plastic containers with dimensions of 27×21×15cm. Each container was covered with a wire mesh to prevent rats from escaping and to ensure proper ventilation. Overhead nipple drinkers and metallic feeding troughs were used to provide water and feed respectively. The metal feeding troughs were fastened to a corner of the containers with bolts and nuts. The containers which housed the rats were randomly placed on metallic shelves. Signs of disease or ill-health were checked before the study and on a daily basis in the course of the experiment. All the containers were cleaned

daily and rats were given ad-lib access to feed and water.

Parameters measured

Yield estimation

One hundred cocoa fruits were collected and weighed. The pods were broken and the seeds were separated from the placenta. The pods, seeds and placenta were then weighed separately. The average fraction of the fruit which the placenta and the seeds constituted was then computed. Using Ghana's average yearly tonnage of cocoa produced from the year 2000 to 2010 (Statistics Research and Information Directorate - Ministry of Food and Agriculture (SRID-MoFA), 2011), the quantity of cocoa placenta disposed of was estimated as:

$$\text{Estimated cocoa placenta yield} = \frac{\text{average fraction of dried placenta from 100 fruits (\%)} \times \text{total cocoa beans exported}}{\text{average fraction of cocoa seeds from 100 fruits (\%)}}$$

Growth performance and economies of production

Feed intake and live weight changes were recorded weekly and served as bases for calculating the daily feed intake, weight gain and the efficiency of utilization of feed. The costs of collecting, transporting and processing were considered in estimating the price of CPM whilst the open market price was used in estimating the cost of all other ingredients used in the study. Feed cost index for the experimental diets was calculated as:

$$\text{Feed cost index (\%)} = \frac{\text{cost of an experimental diet}}{\text{cost of control diet}} \times 100\%$$

The feed cost per 100g weight gain was estimated using the equation:

$$\text{Feed cost/100g wt.gain} = \text{FCR} \times \text{feed cost}$$

Carcass component

On the twenty-eighth day of each trial, all rats were weighed and euthanized for subsequent carcass analyses. The weights of the viscera, heart, kidneys, lungs, spleen, liver, and full and empty gastrointestinal tract (G.I.T) were measured using an electronic scale (KERN & Sohn GmbH, Balingen, Germany). Relative weights of these organs were determined as the percentage of the weight of the organ to the total weight of the rat. All internal organs were physically observed for any signs of abnormality after the rats were eviscerated.

Statistical analysis

Data collected on the growth, economies of production and carcass components of the rats were analyzed using the analysis of variance (ANOVA) procedure of the GenStat Statistical Package (VSN International Limited, 2008). Differences were deemed significant at an alpha level of P < 0.05.

Ethical statement

Due to the absence of ethical policy document on the use of live animals for research at the Kwame Nkrumah University of Science and Technology at the time of this research, the authors adopted the Massey University Code of Ethical Conduct for the use of live animals for research, testing and teaching. MUAEC Code 2014-18 (Amended 2018) Feb.pdf (202 KB)

Results and Discussion

Cocoa placenta yield estimation

Estimates from the Statistics Research and Information Directorate - Ministry of Food and Agriculture (SRID-MoFA) (2011) indicated that Ghana produced a yearly average of 604,545 metric tonnes (MT) of cocoa from 2000 to 2010. This indicates that an estimated average amount of 98403.61MT of cocoa placenta could have been obtained yearly.

Nutrient composition of the dried cocoa placenta meal (CPM)

The results of the proximate composition of CPM showed that it contained 13.20% moisture and almost 15% Crude Protein (CP) on as-fed basis (Table 3). Ether extract (EE), Crude Fibre (CF) and ash were all below 5% whilst almost two-thirds of CPM was the nitrogen free extract (NFE). It was further detected that CPM contains more than 3000 kcal/kg ME.

Health, growth performance and economies of production

The rats showed no signs of ill-health during the entire experimental period and no mortalities were recorded. Physical examination of viscera also indicated that all organs were normal suggesting that none

Table 3: The nutrient composition of dried cocoa placenta meal (CPM)

Proximate component	Moisture	CP	Ash	EE	CF	NFE	ME*
% (As-fed)	13.20	14.88	3.37	3.0	2.38	63.17	3006.91
% (DM)	-	17.14	3.88	3.46	2.74	72.78	3464.51

ME-Metabolizable energy (kcal/kg)

Table 4: Growth performance and economics of production of rats fed CPM with or without XZYMETM

PARAMETER	TREATMENTS				F.pr
	ST ₁ T ₁ (0% CPM)	ST ₁ T ₂ (5% CPM)	ST ₁ T ₃ (10% CPM)	ST ₁ T ₄ (15% CPM)	
Trial 1					
Initial weight, g	75.8	75.8	75.0	76.0	0.99
Final weight, g	169.2	154.0	163.0	143.2	0.61
Mean daily feed intake, g	12.70	11.71	13.89	12.79	0.39
Mean daily weight gain, g	3.34	2.79	3.14	2.40	0.46
FCR	4.01	4.35	4.75	5.82	0.17
Feed cost index ¹	1.00	0.95	0.90	0.86	-
Feed cost/100g wt gain, Gh□	0.59	0.63	0.67	0.80	0.28
Trial 2					
Initial weight, g	58.2	58.2	58.2	57.8	1
Final weight, g	138.2	136.0	148.2	142.8	0.90
Mean daily feed intake, g	10.95	11.77	12.76	13	0.20
Mean daily weight gain, g	2.86	2.78	3.21	3.04	0.84
FCR	4.04	4.33	3.99	4.61	0.67
Feed cost index ¹	1.00	0.96	0.91	0.87	-
Feed cost/100g wt gain, Gh□	0.59	0.60	0.53	0.60	0.74

F.Pr. - probability, 1 not subjected to statistical analysis, □XZYMETM was added at the rate of 250mg per kg of diet for Trial 2 in CPM-containing diets.

Table 5: Effects of CPM with or without XZYMETM on carcass characteristics of rats

PARAMETER	TREATMENTS				F.pr
	ST ₁ T ₁ (0% CPM)	ST ₁ T ₂ (5% CPM)	ST ₁ T ₃ (10% CPM)	ST ₁ T ₄ (15% CPM)	
Absolute weight (g)					
Viscera	35.50	30.90	35.20	29.80	0.37
Kidney	1.49	1.35	1.32	1.29	0.54
Heart	0.62a	0.62a	0.58a	0.45b	0.009
Lung	1.37	1.37	1.56	1.5	0.77
Liver	9.41	6.40	8.68	7.77	0.24
Spleen	1.10	1.05	0.91	1.04	0.80
Full GIT	20.58	17.36	21.33	17.49	0.29
Empty GIT	10.82	9.92	9.93	9.39	0.69
Relative weight (%)					
Viscera	21.08	20.04	22.03	20.87	0.60
Kidney	0.90	0.87	0.81	0.91	0.37
Heart	0.38a	0.41a	0.36ab	0.31b	0.03
Lung	0.83	0.89	0.97	1.06	0.33
Liver	5.52	4.44	5.34	5.44	0.46
Spleen	0.68	0.68	0.57	0.74	0.74

PARAMETER	TREATMENTS				F.pr
	ST ₁ T ₁ (0% CPM)	ST ₁ T ₂ (5% CPM)	ST ₁ T ₃ (10% CPM)	ST ₁ T ₄ (15% CPM)	
Full GIT	12.22	11.23	13.45	12.23	0.32
Empty GIT	6.40	6.49	6.32	6.61	0.97
Absolute weight (g)					
Viscera	31.70	30.70	35.00	31.7	0.68
Kidney	1.27	1.41	1.43	1.26	0.50
Heart	0.52	0.57	0.56	0.48	0.50
Lung	1.32	1.47	1.34	1.48	0.82
Liver	7.48	7.45	8.32	7.66	0.84
Spleen	0.87	1.03	1.02	0.73	0.32
Full GIT	19.67	17.57	21.49	19.33	0.46
Empty GIT	10.32	10.35	10.06	8.92	0.59
Relative weight (%)					
Viscera	23.12	22.55	23.66	22.41	0.71
Kidney	0.91	1.05	0.98	0.89	0.19
Heart	0.37	0.43	0.38	0.34	0.19
Lung	0.97	1.07	0.91	1.06	0.63
Liver	5.40	5.47	5.61	5.37	0.88
Spleen	0.62	0.78	0.71	0.53	0.30
Full GIT	14.41	12.91	14.54	13.67	0.43
Empty GIT	7.59	7.68	6.83	6.30	0.20

F. Pr. – probability

of the diets had any detrimental effects on the rats. There were no significant ($P > 0.05$) differences in the quantities of feed consumed by the rats on the experimental diets in each of the trials (Table 4). Numerical differences were recorded although no clear trends were observed in feed intake values in Trial 1 when no XZYMETM were added to the diets. Even though not significant ($P > 0.05$), daily feed intake increased numerically with the increasing inclusion levels of CPM when XZYMETM was added to the dietary treatments (Trial 2). Differences recorded for weight gain and the efficiency of conversion of feed by the rats in both trials were also statistically similar ($P > 0.05$).

It should be noted that, the partial replacement of maize with CPM resulted in the reduction of feed cost by 5, 10 and 14% in treatments STIT2, STIT3, STIT4 respectively

when compared to STIT1. In trial 2, treatments ST2T2, ST2T3, ST2T4 were 4%, 9% and 13% respectively cheaper than the Control (ST2T1). The cost per 100g weight gain was not significantly different however, numerically, it cost less to raise rats on the Control diet than the CPM-containing diets (Trial 1). When the CPM-containing diets were supplemented with XzymeTM (Trial 2), there were again no significant ($P > 0.05$) differences but numerically the costs involved in weight gain in rats were much more closer to values obtained for animals on the Control diet that was realized in the first trial.

Carcass components

Feeding albino rats with the various CPM-containing diets did not influence the weight of vital organs with the exception of the heart (Table 5). The average weight of

hearts for rats fed the diet containing 15% CPM in trial 1 (STIT4) was significantly ($P < 0.05$) lower than those recorded for rats on all other treatments. Comparing the development of organs to the total weights of the animals, the average weight of the hearts for rats on treatment STIT4 was lower ($P < 0.05$) than those recorded for rats on treatments STIT1 and STIT2. The relative weight of hearts for rats on the experimental diet containing 10% CPM (STIT3) was similar ($P > 0.05$) to those of rats on all the other treatments. In Trail 2, where 250mg of XZYMETM was added to all diets containing CPM, no statistical differences ($P > 0.05$) were recorded in the weights of the vital organs.

Discussion

Ghana is the second leading producer and/ or exporter of cocoa in the world (Asante-Poku and Angelucci, 2013; Nielsen *et al.*, 2007). However, Addy (2000) indicated that cocoa farmers considered cocoa placenta as a waste and had earlier recommended its use in fertilizing soils since it had appreciable quantities of potassium and phosphorus. Later, Frimpong and Awuah (2003) observed that cocoa placenta supports the growth of *Phytophthora palmivora* and as such could be used in the preparation of growth media for research into the black pod disease. However, the yield estimates of cocoa placenta indicated that considerable quantities can be obtained and used in livestock production especially in cocoa growing areas after its nutritive value had been clearly established.

The proximate components of CPM indicate that it contains more CP than maize (NRC, 1998) which it partially replaced in the experimental diets. The ME content of maize, according to the same author NRC (1998), however is higher than that of CPM (3420 vs 3006.91 kcal/kg). Thus, based on these energy levels, it is expected that livestock may consume more CPM than maize to meet their ME requirements. Also a farmer feeding CPM based diets may have to add other high-energy ingredients to make up for this energy deficit.

It is noteworthy that, CPM contains less CP but has more NFE than cocoa bean cake which had earlier been studied by Odunsi and Longe (1995).

Earlier, Adamafio (2013) explained that, the theobromine in cocoa and cocoa by-products is toxic and detrimental to animal growth. He further proposed that it would be better to use limited quantities of cocoa by-products in the diets of livestock. Again, Alexander *et al.* (2008) indicated that feeding high levels of cocoa by-products to livestock may elicit responses such as diarrhoea, growth retardation, kidney and liver damage and ultimately death. The fact that no ill-health or mortalities were recorded in these experiments suggests that CPM may not contain enough secondary metabolites which can be lethal to rats. Recently, Cruz *et al.* (2015) reported that sun-drying of cocoa reduces the levels of its phenolic compounds but indicated that methylxanthines (such as caffeine and theobromine) contents may vary depending on the variety of cocoa. Emiola *et al.* (2011) however, reported that feeding diets containing up to 30% of sundried cocoa bean shells to poultry negatively affected growth and reproduction. It has been observed that, feeding up to 15% cocoa by-products, as was the case in this study, may not elicit any detrimental effects on monogastric farm animals (Odunsi and Longe, 1995).

Odunsi and Longe (1995), reported lower feed intake values when pullets were given diets containing varying levels of cocoa bean cake. The authors attributed this depression in feed intake to the theobromine found in cocoa and its by-products. It is worth mentioning that, Odunsi and Longe (1995) fed diets containing as much as 40% cocoa bean cake (CBC) whilst the current study used a maximum CPM level of 15%. The reduction in the cost of the CPM-based diets could be attributed to the fact that agro-industrial by-products such as cocoa placenta are of relatively low demand and as such, farmers may pay nothing or meager sums of money to acquire them (Okai and Boateng, 2007). This low feed cost notwithstanding, the fact that the cost per weight gain improved marginally upon the addition of the exogenous

enzyme complex may be an indication that CPM contains anti-nutrients which were inactivated.

An earlier study by Odunsi and Longe (1995) resulted in significantly lower heart weights when pullets were fed CBC-based diets. The fact that heart weights were similar ($P > 0.05$) after the probiotic-enzyme complex was added to the CPM diets is an indication that CPM contains certain secondary metabolites which are scavenged by the probiotic-exogenous enzyme complex. Contrary to this, Emiola *et al.* (2011) reported reduced spleen and kidney weights when laying hens were fed cocoa bean shell-based diets and attributed this to the harmful secondary metabolites that may be in cocoa by-products. Thus the authors recommended treatments which will inactivate these metabolites if such products are to be used in livestock feeding.

Conclusions and Recommendations

This study showed that Ghana produced an estimated yearly average of 98,403.61MT of cocoa placenta from the year 2000 to 2010 which can be used in feeding livestock especially those in cocoa growing areas. Feeding diets containing up to 15% CPM with or without XZYME TM had no adverse effects on the health, growth performance and carcass characteristics of experimental rats. It is however recommended that, further trails be carried out to identify the anti-nutrients present therein and ways of reducing them. Also more studies should be carried out to determine the effects of CPM on monogastrics like poultry and pigs.

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GROWTH AND CARCASS YIELD OF RABBITS IN TWO HOUSING TYPES ADMINISTERED AQUEOUS EXTRACT OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)

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Abstract

A total of ninety-six rabbits consisting of forty-eight males and forty-eight females of mixed breeds, aged six weeks with an average weight of 620 g were used for the study. The rabbits were grouped into housing types of steel and wooden hutches, sex and four levels of inclusion (0, 5, 10 and 15 ml) of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) per litre of water. The rabbits were maintained on an ad-libitum concentrate diet for a 10-week experimental period. At the end of the 10th week, a rabbit was selected from each replicate of the rabbit bucks for carcass yield evaluation. The data generated were subjected to a Completely Randomized Design. Results showed that rabbits housed in steel hutches had a higher ($p < 0.05$) mortality (18.75 %) than those in wooden hutches (4.17 %). Bucks consumed more ($p < 0.05$) water (308.47 ml) per day than the growing does (276.52 ml). Bucks housed in steel hutches had bigger ($p < 0.05$) lungs (0.84 %) than those in wooden hutches (0.67 %). The bucks housed in steel hutches had more ($p < 0.05$) ash in the meat (1.25 %) than those housed in wooden hutches (1.14 %). The study showed that mortality in rabbits can be minimized when housed in wooden hutches as compared to steel hutches and that an aqueous extract of oyster mushroom can replace multivitamins and antibiotics as a growth promoter for rabbits.

Keywords: carcass yield, rabbits, growth performance, housing types, aqueous extract, oyster mushroom,

CROISSANCE ET RENDEMENT EN VIANDE DES LAPINS RECEVANT DES EXTRAITS AQUEUX DE PLEURETES (*PLEUROTUS OSTREATUS*) DANS DEUX TYPES DE LOGEMENTS

Résumé

Au total, quatre-vingt-seize lapins comprenant quarante-huit mâles et quarante-huit femelles de races mixtes, âgés de six semaines et pesant en moyenne 620 g, ont été utilisés pour l'étude. Les lapins ont été groupés dans des types de logement de clapiers en acier et en bois, par sexe, et ont reçu quatre niveaux d'inclusion (0, 5, 10 et 15 ml) d'extrait aqueux de pleurote (*Pleurotus ostreatus*) par litre d'eau. Les lapins ont été maintenus sous un régime concentré ad-libitum pendant une période expérimentale de 10 semaines. À la fin de la dixième semaine, un lapin a été sélectionné dans chaque répétition de mâles pour l'évaluation du rendement en viande. Les données générées ont été soumises à un schéma complètement aléatoire. Les résultats ont montré que les lapins logés dans des clapiers en acier avaient une mortalité (18,75%) plus élevée ($p < 0,05$) par rapport à ceux des clapiers en bois (4,17%). Les mâles consommaient plus ($p < 0,05$) d'eau (308,47 ml) par jour que les femelles en croissance (276,52 ml). Les mâles logés dans les clapiers en acier avaient des poumons plus grands ($p < 0,05$) (0,84%) par rapport à ceux logés dans les clapiers en bois (0,67%). La viande de mâles logés dans les clapiers en acier avait plus ($p < 0,05$) de cendres (1,25%) que celle des animaux logés dans les clapiers en bois (1,14%). L'étude a montré que la mortalité des lapins peut être minimisée lorsqu'ils sont logés dans des clapiers en bois par rapport aux clapiers en acier, et qu'un extrait aqueux de pleurote peut remplacer les multivitamines et les antibiotiques comme facteur de croissance des lapins.

Mots-clés: rendement en viande, lapins, performances de croissance, types de logement, extrait aqueux, pleurote

Introduction

It is widely recognised that rabbit production hinges on housing and management (Mailafia *et al.*, 2010). Poor housing is common in the less developed countries including Nigeria and is a limiting factor to growth performance of rabbits, carcass yield and meat quality (McNitt *et al.*, 2000; Dal Bosco *et al.*, 2000; Dalle Zotte, 2002; Pla, 2008; Chodova *et al.*, 2014) and reproductive behaviour of rabbits (Marai & Rashwan, 2003). The steel hutch, one of the major housing types for rabbit production has the merits of easy to clean, disinfect and prevent unsanitary conditions that aid and promote the spread of bacterial diseases which cannot be provided in wooden hutches (Lebas, 1997; Shaeffer and Kime, 2008). However, a greater incidence of bacterial diseases, especially respiratory problems such as pneumonia, chronic respiratory and pasteurella diseases might be more prominent with the usage of steel hutches as a result of uncontrolled air flow.

There is presently an increasing effort among livestock farmers and animal scientists including rabbit farmers to eliminate and replace conventionally used antibiotics and multivitamins with natural plant products and sources such as phytobiotics as active substances in order to improve on growth performance, the health status of rabbits and to produce organic rabbit meat (Gugolek *et al.*, 2011). This has necessitated research on cheaper and more affordable alternatives to the use of conventional antibiotics and multivitamins. The use of mushrooms as phytobiotic compounds in rabbit production and an important food item in human health, nutrition and disease prevention is on the increase (Khan *et al.*, 2008). *Pleurotus* species, as one of the valuable and edible species of mushroom, occupies the third place in the worldwide production of edible mushrooms, after *Agaricus bisporus* and *Lentinula edodes* (Chang, 1999). It is reported to be the second most important mushroom in production in the world and it accounts for 25% of the total world production of cultivated mushrooms and is the most popular species in

Nigeria (Adejoye *et al.*, 2006; Fakoya, 2011).

The fruit bodies of *Pleurotus* species are a good source of non-starchy carbohydrates, dietary fibre (that can help in reducing plasma cholesterol), are rich in protein, contain an abundance of most of the essential amino acids, minerals (calcium, phosphorus, iron) and vitamins of B group (thiamin, riboflavin and niacin) and folic acid (Bano *et al.*, 1988; Manzi *et al.*, 1999; Sadler, 2003). The reported high nutritive value of *Pleurotus* species (Sadler, 2003; Çağlarımak, 2007; Alam *et al.*, 2008; Marshall and Nair, 2009) informed the study on the growth and carcass yield of rabbits in two housing types administered an aqueous extract of oyster mushroom (*Pleurotus ostreatus*).

Materials and Methods

The experiment was carried out at the Rabbit Unit of the Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The experimental site is located in the rain forest vegetation zone of south-western Nigeria on latitude 7° 10" N, longitude 3° 2" E and altitude 76m above sea level. The climate is humid with a mean annual rainfall of 1037 mm and mean temperature and humidity of 34.7°C and 83%, respectively.

A total of ninety-six rabbits consisting of forty-eight male rabbits and forty-eight female rabbits of mixed breeds, aged six weeks with an average weight of 620 g, were used for the study. The rabbits were grouped on uniform average weight basis into two different housing types of steel and wooden hutches of forty-eight rabbits each. Rabbits in each housing type were further divided into male and female groups of twenty-four rabbits each to check for the effect of sex. Each group was subjected to one of four levels of inclusion (0, 5, 10 and 15 ml) of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) per litre of water. Six rabbits were assigned to each treatment group and each group was replicated thrice with two rabbits per replicate. The rabbits were adapted to the new environment prior to the start of the experiment for a period of two weeks. A uniform feed composition of concentrate diet

was fed to all the rabbits as shown on Table I over the 10 weeks experimental period. Multi-vitamins and antibiotics were administered in the drinking water in the course of the experiment only to rabbits with no inclusion level of aqueous extract of oyster mushroom. The multi-vitamins and antibiotics used was Neotreat WSP® from Kepro, Netherlands. The aqueous extract of oyster mushroom levels was administered to the rabbits thrice per week for a duration of eight weeks. The administration of aqueous extract of oyster mushroom levels were withdrawn for the last two weeks of the experiment. The rabbits were housed in the steel and wooden hutches individually and each hutch had a dimension of 60 cm x 60 cm x 50 cm. Feeders and drinkers were provided in the hutches with unrestricted access to feeds and fresh clean drinking water. The rabbits were managed under natural environmental conditions. Routine management procedures were adhered to in the course of the experiment.

An aqueous extract of oyster mushroom (*Pleurotus ostreatus*) was prepared with the use of hot water extraction procedure by extending the boiling process of the mushrooms so as to fully extract the polysaccharides which are considered to be medicinal out of the mushroom cell wall. The oyster mushrooms were cooked for a duration of twenty (20) minutes at 57.2 °C at the ratio of 500 g of oyster mushroom to 1 litre of water. The newly formed extracts were then cooled and strained off the mushrooms with the aid of a sieve. The extracts were kept in a dark-coloured recipient (to prevent photolysis due to light penetration) and then stored in the refrigerator until needed.

Data were taken on a daily basis for feed and water intakes, and on weekly basis for weight gain. Feed conversion ratio, feed to water ratio and mortality were calculated.

$$\text{Total feed intake (g)} = \text{Total feed given (g)} - \text{Total feed left over (g)}$$

$$\text{Average daily feed intake (g)} = \frac{\text{Total feed intake (g)}}{\text{Total number of days of the experimental period}}$$

$$\text{Total water intake (ml)} = \text{Total water given (ml)} - \text{Total water left over (ml)}$$

$$\text{Average daily water intake (ml)} = \frac{\text{Total water intake (ml)}}{\text{Total number of days of the experimental period}}$$

$$\text{Body weight gain (g)} = \text{Final body weight (g)} - \text{Initial body weight (g)}$$

$$\text{Daily body weight gain (g)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Total number of days of the experiment period}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed intake (g)}}{\text{Total body weight gain (g)}}$$

$$\text{Feed to water ratio} = \frac{\text{Total feed intake (g)}}{\text{Total water intake (ml)}}$$

$$\text{Mortality} = \frac{\text{Number of dead rabbits} \times 100}{\text{Total number of rabbits}}$$

At the end of the 10th week of the experiment, one rabbit was selected from each replicate of the rabbit bucks for carcass evaluation. Feed was withdrawn from the rabbits at 12 hours before slaughtering in order to empty their gastro intestinal tracts (GIT) so as to reduce the variability in body weight due to intestinal content. The rabbits selected for the carcass evaluation were weighed prior to slaughtering. The rabbits were later stunned, bled, scalded and then eviscerated. The carcass weight and dressing percentage of the rabbits were determined and recorded. The carcass was cut into wholesale parts (fore limb, hind limb, loin, chest, back, head, neck and tail) and then weighed with a sensitive electronic weighing scale. The pH of the meat (thigh muscle) was also determined. The weight of the kidney, liver, lung and heart were also taken. Individual weights were noted for each rabbit and then expressed as a percentage of live weight.

The meat samples were analysed (AOAC, 2016) to determine the dry matter content by oven drying, ether extract by Soxhlet Analysis, crude protein by Kjeldal Method, crude fibre by TCA Method, ash by Muffle Furnace and nitrogen free extract. The meat samples were cut out from the thigh muscle of the rabbit bucks used for carcass yield determination.

The data generated were subjected to a Completely Randomized Design. The rabbits were grouped into two housing types (wooden and steel hutches), sex (male and female) and four levels (0, 5ml, 10ml and 15ml) of inclusion of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) per litre of drinking water. Significantly ($P < 0.05$) different means were separated using Tukey Test as contained in Minitab® 17.1.0 2013. The data generated were analysed with the use of General Linear Model (GML) of the same package.

Results

The effects of housing types and sex on the growth performance of rabbits are

shown on Table 2. The effect of the housing types was obtained only on the mortality of the rabbits. The rabbits housed in steel hutches had a higher mortality (18.75 %) than the rabbits housed in wooden hutches (4.17 %) (Table 2). The sex affected the water intake per day of the rabbits. The male rabbits had a higher water intake per day (308.47 ml) than the female rabbits (276.52 ml) (Table 2).

Table 3 shows the effects of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) levels on the growth performance of the rabbits. The aqueous extract of oyster mushroom levels did not affect the growth performance of the rabbits (Table 3).

The effects of housing types and oyster mushroom extract levels on carcass characteristics of rabbit bucks are shown in Table 4. The housing types (steel hutches and wooden hutches) had effects on the lungs of the growing rabbits. Rabbit bucks housed in steel hutches had bigger lungs (0.84 %) than the rabbit bucks housed in wooden hutches (0.67 %) (Table 4). In the same vein, the varying levels of aqueous extract of oyster mushroom affected the necks of the rabbit bucks. Rabbits

Table 1: Gross composition of concentrate diet for the rabbits

Feed ingredients	Composition (%)
Maize	35.00
Wheat offal	20.00
Rice bran	17.00
Palm kernel cake	12.00
Soya-bean meal	10.00
Bone meal	2.00
Fish meal (65%)	2.00
Limestone	1.00
Salt	0.50
Premix (Growers)	0.50
Total	100.00
Calculated Analysis	
ME (MJ/kg)	10.53
Crude protein (%)	16.64
Crude fibre (%)	6.68

Table 2: Effects of housing types and sex on growth performance of rabbits

Parameters	Housing types		Sex		SEM	P value
	Steel hutch	Wooden hutch	Male	Female		
Initial weight (g/rabbit)	772.92	776.04	756.25	792.71	12.40	0.12
Final weight (g/rabbit)	1530.21	1593.75	1547.92	1576.04	26.40	0.46
Weight gain (g/rabbit)	757.29	817.71	791.67	783.33	24.10	0.81
Average daily weight gain (g/rabbit)	10.82	11.68	11.31	11.19	0.35	0.81
Total feed intake (g/rabbit)	5222.88	5265.90	5356.50	5132.27	88.10	0.08
Average daily feed intake (g/rabbit)	74.61	75.23	76.52	73.32	1.26	0.08
Feed conversion ratio	7.09	6.52	6.95	6.67	0.23	0.39
Water intake per day (ml/rabbit)	297.95	287.05	308.47a	276.52b	10.50	0.04
Feed to water intake ratio (g/ml)	1.00:4.00	1.00:3.81	1.00:4.03	1.00:3.78	0.13	0.18
Mortality (%)	18.75a	4.17b	14.58	8.33	4.42	0.33

ab means in the same row with different superscripts are significantly different ($P < 0.05$). SEM = Standard Error of Means

Table 3: Effects of aqueous extract of oyster mushroom (Pleurotus ostreatus) levels on growth performance of rabbits

Parameters	Mushroom extract levels				SEM	P value
	0	5ml	10ml	15ml		
Initial weight (g/rabbit)	791.67	752.08	785.42	768.75	17.50	0.39
Final weight (g/rabbit)	1537.50	1518.75	1622.92	1568.75	37.30	0.24
Weight gain (g/rabbit)	745.83	766.67	837.50	800.00	34.10	0.27
Average daily weight gain (g/rabbit)	10.65	10.95	11.96	11.42	0.49	0.27
Total feed intake (g/rabbit)	5245.75	5220.88	5415.29	5095.63	125.00	0.36
Average daily feed intake (g/rabbit)	74.94	74.58	77.36	72.79	1.78	0.36
Feed conversion ratio	7.20	7.00	6.62	6.42	0.33	0.34
Water intake per day (ml/rabbit)	311.08	279.37	296.99	282.55	14.80	0.42
Feed to water intake ratio (g/ml)	1.00:4.15	1.00:3.75	1.00:3.83	1.00:3.90	0.18	0.46
Mortality (%)	4.17	12.50	12.50	16.67	6.25	0.56

SEM = Standard Error of Means

Table 4: Effects of housing types and aqueous extract of oyster mushroom (*Pleurotus ostreatus*) levels on carcass characteristics of rabbit bucks

Parameters	Housing types				Mushroom extract levels					SEM	P value	P value
	Steel hutch	Wooden hutch	SEM	P value	0	5ml	10ml	15ml				
	1525.00	1437.50	42.40	0.18	1550.00	1487.50	1450.00	1437.50				
Live weight (g/rabbit)	1091.00	1007.20	35.7	0.13	1085.60	1072.20	1027.40	1011.20	59.90	0.57		
Carcass weight (g/rabbit)	71.49	70.10	0.91	0.31	69.91	72.10	70.85	70.32	50.50	0.70		
Dressing percentage	6.68	6.76	0.05	0.27	6.75	6.63	6.80	6.69	1.29	0.66		
pH									0.07	0.34		
Cut-up parts (%)												
Head	8.77	9.10	0.24	0.36	9.28	8.71	8.60	9.16	0.34	0.46		
Fore limb	8.43	7.47	0.44	0.16	7.78	8.99	6.80	8.23	0.62	0.17		
Hind limb	16.37	15.81	0.36	0.31	15.92	16.66	15.25	16.54	0.51	0.26		
Chest	7.77	8.42	0.62	0.48	7.29	8.03	8.17	8.90	0.88	0.66		
Loin	14.68	15.37	0.98	0.63	15.51	14.19	15.55	14.84	1.39	0.88		
Back	12.26	10.86	1.07	0.39	10.83	11.91	13.82	9.68	1.52	0.33		
Neck	2.56	2.50	0.12	0.72	2.60 ^{ab}	3.12 ^a	1.99 ^b	2.41 ^{ab}	0.17	0.01		
Tail	0.66	0.57	0.05	0.33	0.71	0.51	0.68	0.55	0.08	0.26		
Organs (%)												
Lungs	0.84 ^a	0.67 ^b	0.05	0.05	0.68	0.73	0.82	0.81	0.07	0.51		
Heart	0.27	0.27	0.02	0.97	0.25	0.26	0.26	0.30	0.03	0.77		
Kidney	0.75	0.75	0.03	0.97	0.65	0.80	0.73	0.81	0.05	0.17		
Liver	2.67	2.55	0.11	0.48	2.52	2.84	2.58	2.51	0.16	0.46		

^{ab} means in the same row with different superscripts are significantly different ($P < 0.05$)

SEM = Standard Error of Means

Table 5: Effects of housing types and aqueous extract of oyster mushroom (*Pleurotus ostreatus*) levels on meat proximate composition of rabbit bucks

Parameters	Housing types		Mushroom extract levels					SEM	P value
	Steel hutch	Wooden hutch	0	5ml	10ml	15ml			
Dry matter (%)	28.66	28.59	28.06	28.08	29.47	28.88	0.48	0.19	
Crude protein (%)	25.01	25.25	24.66	24.50	25.76	25.60	0.46	0.20	
Ether extract (%)	2.40	2.20	2.28	2.36	2.46	2.09	0.20	0.61	
Ash (%)	1.25a	1.14b	1.12	1.23	1.25	1.19	0.05	0.28	
Nitrogen-free extract (%)	71.34	71.41	71.94	71.92	70.53	71.12	0.48	0.19	

^{ab} means in the same row with different superscripts are significantly different ($P < 0.05$)
SEM = Standard Error of Means

on provided with 5 ml of aqueous extract of oyster mushroom per litre of drinking water had the biggest necks (3.12 %) while the rabbit bucks on 10 ml of aqueous extract of oyster mushroom per litre of drinking water had the smallest necks (1.99 %) (Table 4).

The effects of housing types and aqueous extract of oyster mushroom levels on meat proximate composition of rabbit bucks are shown on Table 5. housing types (steel hutch and wooden hutch) had effects on the ash content of the meat proximate composition. The ash content (1.25 %) of the meat proximate composition of the rabbit bucks housed in steel hutches had a significantly higher value than the ash content (1.14 %) of the meat proximate composition of the rabbit bucks housed in wooden hutches (Table 5).

Discussion

The study on rabbits housed in steel hutches and wooden hutches showed that housing types did not affect the growth rate, feed and water intakes. These results support the findings of previous studies that showed that housing did not significantly affect the growth rate and productive performance of rabbits (Luzi *et al.*, 2003; Verga *et al.*, 2004). The higher mortality recorded from rabbits kept in steel hutches may have been due to the inability of the rabbits to quickly adapt to the steel hutches as most commercial rabbit farms in Nigeria uses wooden hutches. Furthermore, the battery cage system cannot be insulated and rabbits are more liable to respiratory ailments if air flow is not controlled (Lebas, 1997).

The sex of the rabbits affected their daily water intake. The male rabbits had a higher water intake per day (308.47 ml) than the female rabbits (276.52 ml) (Table 2). The sex of the rabbits did not influence their growth performance, confirming the reports of Oliveira and Lui (2006); Laxmi *et al.* (2009); Pinheiro *et al.* (2012) and Ayo-Ajasa *et al.* (2014). This result also agrees with the investigations of Rollins *et al.*, 1963; Vogt, 1979; Lukefahr and Ozimba, 1991; Ozimba and Lukefahr, 1991) who reported that unlike in most domestic livestock

species, sex does not strongly influence growth performance and carcass characteristics in rabbits.

The similar results from the growing rabbits on varying levels of aqueous extract of oyster mushroom showed that the rabbits performed as well as rabbits on conventional multivitamins and antibiotics and this proves that aqueous extract of oyster mushroom can replace conventional multivitamins and antibiotics as a growth promoter for rabbits. This finding supports that of Sogunle *et al.* (2016) who reported no significant differences on growth performance of broiler chicken with different levels of Oyster mushroom extract.

The results of this study indicated that carcass yield, dressing percentage, pH and cut-out parts characteristics of rabbits would not be affected when rabbits are kept under different housing types (steel and wooden hutches). This might be due to the fact the rabbits were fed on the same diet and were therefore on the same plane of nutrition. This agrees with the study by Pinheiro *et al.* (2011) who reported that housing systems had no effect on the dressing out percentage of rabbits and also with the findings of Trocino *et al.* (2004) that carcass quality were unaffected by housing system. However, the results differed with those of other studies indicated that housing systems can affect body carcass traits (Pla, 2008). The variation in the size of the lungs between the rabbits kept in steel hutches and wooden hutches could be attributable to their health status which was corroborated by the higher incidence of respiratory diseases among rabbits kept in steel hutches. This may have caused the enlargement of the lungs in response to the emergence of these diseases and the incidence of these respiratory diseases was corroborated by Lebas (1997) who reported that rabbits are more liable to respiratory ailments if air flow is not controlled.

The study showed that the proximate composition of meat from rabbits is not influenced by the housing types except for the ash content of the meat. On the other hand, oyster mushroom extract levels did not influence any of the parameters considered.

There was no evidence from the results of this study that substituting the use of multivitamins and antibiotics for aqueous extract of oyster mushroom will improve the nutritive value of the meat from rabbits.

Conclusion

The study showed that mortality in rabbits can be minimized when housed in wooden hutches as compared to steel hutches. Also, aqueous extract of oyster mushroom (*Pleurotus ostreatus*) can replace multivitamins and antibiotics as a growth promoter for rabbits.

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ESTABLISHMENT OF NATIONAL LIVESTOCK DATABANK FOR GENETIC IMPROVEMENT PROGRAMMES IN ZAMBIA

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Abstract

This paper examines the importance and need for livestock data, current practices and challenges in its collection and the different data management systems in use in Zambia. The impending introduction and deployment of the ARIS2 in collaboration with AU-IBAR, under the Animal Resources Data Management Platform, makes it important for practitioners and policy makers to have a re-look at the opportunities for livestock data capture at source and propose measures to populate the new data management system. The need for the current different data management systems to be linked in order to share information and feed the ARIS2 was emphasized. This paper also brings to the fore, the unique data requirements for livestock genetic improvement programmes. Such minimum requirements include the unique identification of each animal, morphometric characteristics, production parameters and carcass qualities. Efficient and effective collection, storage, linkage and sharing that will make for a good data analysis and reporting system are highlighted.

Keywords: animal identification, data management, genetic resources, genetic improvement

ÉTABLISSEMENT DE LA BANQUE DE DONNÉES NATIONALE DE L'ÉLEVAGE POUR LES PROGRAMMES D'AMÉLIORATION GÉNÉTIQUE EN ZAMBIE

Résumé

Le présent document examine l'importance et la nécessité des données sur l'élevage, les pratiques actuelles, les difficultés rencontrées dans la collecte de données et les différents systèmes de gestion de données utilisés en Zambie. L'introduction et le déploiement imminents d'ARIS2 en collaboration avec l'UA-BIRA, dans le cadre de la Plateforme de gestion des données sur les ressources animales, obligent les praticiens et les responsables de l'élaboration des politiques de réexaminer les possibilités de saisie des données sur l'élevage à la source et de proposer des mesures pour alimenter le nouveau système de gestion des données. La nécessité de relier les différents systèmes de gestion de données actuels afin de partager les informations et d'alimenter le système ARIS2 a été soulignée. Ce document met également en évidence les besoins uniques en données pour les programmes d'amélioration génétique des animaux d'élevage. Ces exigences minimales incluent l'identification unique de chaque animal, ses caractéristiques morphométriques, ses paramètres de production et les qualités de sa carcasse. L'étude met en évidence la collecte, le stockage, l'établissement de liens et le partage efficaces et efficaces qui permettront un bon système d'analyse des données et de production de rapports.

Mots-clés : identification des animaux, gestion des données, ressources génétiques, amélioration génétique

Introduction

Livestock productivity in most African countries is reported to be low. The low productivity is generally as a result of poor management practices, poor feeding regime, disease outbreaks and lack of genetic improvement. Although a lot of efforts are being deployed to address some of these challenges, not much could be said about the genetic improvement of the animals. Livestock genetic improvement programmes are hinged on the animal breeding model where a number of sires are mated to a number of dams with each dam producing a number of offspring. This invariably will lead to the collection of a large data set over a long period of time and across farms to determine genetic parameters for use in devising breeding programmes. Hence the development of National Livestock data banks is essential if we are to improve the genetic worth of animals in the developing countries.

Inadequacies and constraints of livestock data capture

There are a number of inadequacies noted with livestock data collection. Hurrell (1957), observed that the capturing of livestock data is fraught with a number of difficulties because it changes daily given new births, deaths, slaughter, growth, age and market dynamics. Notwithstanding the above, livestock data collection in developing countries has often been dogged with issues of insufficiency, scanty and incomplete data, irregular collection, infrequent data collection, untimely data collection and limited coverage of data collection (Pica-Ciamarra *et al.*, 2014). The above are attributed to constraints of the human capital requirements for the exercise; financial outlay and the robustness of the data management systems. All these impact negatively on the quality dimensions of data which include, relevance, accuracy, credibility, timeliness, accessibility, interpretability and coherence as observed by Goma and Daka, 2017, (citing the United Nations Statistical Commission).

Sources of livestock data capture in Zambia

Most often than not, Government through the Central Statistics Office (CSO, 2006; 2014) is the principal agency in the drive for livestock data capture because it is deemed a public good. This is done using a number of deliberate policies such as:

- *Population and housing census* - Mostly government driven and deemed as public goods. It is supposed to be undertaken after every 10 years, funds permitting. Survey questions relating to livestock are nevertheless often very few.
- *Annual post-harvest survey* - Government undertaken every year to forecast crop harvests. The information collected on livestock are equally few touching on the numbers raised, value and services received.
- *Livestock census* - This is a specialized livestock census similar to a population census in principle but is rarely carried out in Zambia. From personal interviews, it has not been carried out in the last 30 years' hence the information on livestock numbers are derived from national households, agricultural and post-harvest farm surveys.
- *Farm animal breed survey* - This is an equally specialized survey carried out to characterize the farm animal breeds in Zambia. It was partly funded by FAO and the Government of Zambia. The first and only survey was carried out in 2001/2. It falls under the Animal genetic resources global plan of action. Specialized livestock surveys are rarely undertaken (Ministry of Agriculture and Cooperatives, 2002)
- Other instances of livestock data capture by government include the incidence of disease outbreaks occasioning vaccination and dipping campaigns. Slaughter houses (abattoirs) run by Local Councils which falls under the Ministry of Local Government and Housing but at the same time are regulated under the Veterinary Acts, also capture data on animals taken for slaughter such as the numbers slaughtered per day

and disease status.

- The government also has the opportunity to collect livestock data from the various established livestock breeding centers for beef and dairy cattle, goats and sheep in various parts of the country. Individual researchers or Research organizations (National Institute of Scientific and Industrial Research (NISIR), National Artificial Insemination Services (NAIS), Central Veterinary Research Institute (CVRI), Golden Valley Research Trust (GART), Livestock Development Trust (LDT), Agricultural Training Institutions and Universities) also generate livestock data depending on the various research projects being investigated.
- Non-Governmental organizations (Herdbook Society of Zambia, Heifer International, SNV, Oxfam, commodity based Associations such as for poultry, beef, dairy, goats and sheep) and farmers' (smallholder farmers, commercial farmers) also collect and keep data on livestock production for their operations and management practices.

It is instructive to note that there were no attempts at collecting data on any livestock production parameters by the Government of Zambia. (Goma and Daka, 2017) are of the view, that a separate livestock survey conducted annually would provide the much needed data to properly measure the performance of the livestock sector in Zambia and this would lead to the formulation of relevant policies that would promote growth of the sector. Decisions would thus be based on evidence.

Opportunities for Zambia National data capture

It is quite clear from the above that while several bodies gather and store livestock production data, there is no known coordinated Livestock production databank for the various animal genetic resources. Nevertheless, there exist opportunities for livestock production data capture and data management in Zambia.

While it is appreciated that there are pockets of data collection and management by most players in the livestock sector, there is need to either aggregate the various databases or establish an integrated system which links the various data sources to form the National Livestock Production database.

Table I below, gives a profile of the various types of data being generated or that can be generated and by which organization. It also highlights the implications for synergy among the different players in the livestock sector. The table indicates overlaps in the functions and responsibilities with regards to the livestock sector of a number of Ministries highlighting the need for cooperation and coordination among them to ensure success of the efforts.

For instance, the Universities and NISIR are under the Ministry of Higher Education; Slaughter houses fall under the Ministry of Local Government; CSO is under the Ministry of Finance; Colleges of Agriculture are under the Ministry of Agriculture. The Agricultural Training institutions and Research stations fall under both the Ministry of Agriculture and Ministry of Fisheries and Livestock.

There is therefore a need for the country to relook at the way livestock production data is being captured and establish an integrated system which links the various data sources. It would thus be necessary to have a Lead Agency to serve as Node for data collection for each livestock data indicator in the various categories for coordination purposes, assignment of appropriate levels of rights & privileges and also serve as clearing houses.

It would also be necessary to leverage on related Livestock Acts and Policies. The Policies that can aid data collection include the following:

- Animal Health Act 2010 (under revision)
- Animal Identification Act of 2011
- The Livestock Development Policy (under development)
- Animal Breeding Policy (under development)

The Animal Identification Act of 2011 which provides for each animal to be identified by means of an owner's registered mark (including branding) does not go far enough for each animal (especially for ruminants and pigs) to have its own unique identification separate from all other animals similarly branded, belonging to the same owner. While this could be important in dealing with herds or flocks reared in the same environment, it may not be appropriate for the selection of individuals with genetic superiority or high breeding value. This has implications for Animal Identification Registration and Traceability in the country.

Table 1: Livestock indicators, data sources and stakeholders

No	INDICATOR	DATA SOURCES	STAKEHOLDERS
1	Livestock numbers (species, sex, age) Breeds or Strains or Varieties Breeding population	<ul style="list-style-type: none"> • Livestock census • Post-Harvest Survey • Commodity Association • Stock registers 	<ul style="list-style-type: none"> • Central Statistical Office (CSO) • Department of Veterinary Services • Department of Livestock Development • Zambia National Farmers Union • Commodity Associations • Training, R and D
2	Livestock Marketing Information (Prices, Numbers marketed -domestic and imports and exports , value of sales)	<ul style="list-style-type: none"> • Post-Harvest Survey • Veterinary/Livestock Development field staff • AMIC (Agriculture Marketing Information Centre) • Ministry of Local Government • Commodity Associations • Training , R and D • Chamber of Commerce • ASYCUDA 	<ul style="list-style-type: none"> • Central Statistical Office (CSO) • Department of Veterinary Services • Department of Livestock Development • Zambia National Farmers Union • Department of Agri Business • Data Providers (Slaughter facilities, processing plant), • Zambia Revenue Authority • Ministry of Trade, Commerce and Industry • Commodity Associations
3	Livestock Production Performance (Conception rate, Calving %, Calving Interval, Body weights, Carcass percentage, Milk production etc.)	<ul style="list-style-type: none"> • Breeding Research Stations, • Artificial Insemination Centres • Training, R and D • Commodity Associations • Farmers 	<ul style="list-style-type: none"> • Breeding Research Stations, • Artificial Insemination Centres • Training, R and D • Department of Veterinary Services • Department of Livestock Development • Livestock Farmers
4	Livestock Feed Produced by Type	<ul style="list-style-type: none"> • Industrial Production Statistics • Feed producers • Commodity Associations 	<ul style="list-style-type: none"> • CSO • Ministry of Fisheries and Livestock • Ministry of Agriculture • Millers Association of Zambia • Feed Manufacturers

No	INDICATOR	DATA SOURCES	STAKEHOLDERS
5	Measures of Disease occurrence Prevalence/ Mortality/ Morbidity, Incidence	<ul style="list-style-type: none"> • Veterinary Field Staff • Post-Harvest Survey • Training, R and D • Processing plants • Private Animal health facilities 	<ul style="list-style-type: none"> • Department of Veterinary Services • Training, R and D • CSO • Private Veterinarians • Ministry of Local Government • Farmer Associations • Ministry of Health
6	Disease Control measures Treatments, Stamping out, Deworming, Dipping	<ul style="list-style-type: none"> • Veterinary Field Staff • Post-Harvest Survey • Private Animal health facilities • Zambia Medicines Regulatory • Agroveter drugs Suppliers 	<ul style="list-style-type: none"> • Department of Veterinary Services • Farmers • Private Veterinarians
7	Infrastructures and Machinery Livestock centres/Dipping tanks/Abattoirs	<ul style="list-style-type: none"> • Department of Veterinary Services • Department of Livestock Development • Veterinary Council of Zambia 	<ul style="list-style-type: none"> • Department of Veterinary Services • Department of Livestock Development • Veterinary Council of Zambia

Adapted from Goma and Daka (2017)

Data requirement for Livestock Genetic Improvement

Under Strategic priority area I of the FAO Global Plan of Action for Animal Genetic Resources, it is incumbent for each country to carry out an Inventory of the various farm animals; characterize each species in terms of morphometric measurements, production characteristics and adaptive traits. The inventory helps to monitor trends, associated risks associated and the in determining conservation priorities and strategic breeding programmes. (FAO 2007).

Of the listed Livestock indicators or segments of livestock data, the two most important for Livestock genetic improvement programmes are the Pedigree and Production performance data sets. It is a costly exercise taking measurements, recording and storing data on each animal. The production performance data sets need to be populated not only from the research stations but also from both

smallholders and commercial farmers; cutting across farms and research stations. Without these two data sets, any livestock genetic improvement would be subjective and not measureable.

Few reports exist in literature on the phenotypic characterization of the animal genetic resources of Zambia. The Farm animal breed survey carried out in 2011/2012 provided an insight into the existing. Besides this report, there were also reports by (Simbaya, 2011) and (Musimuko, 2014) on the phenotypic characterization of cattle breeds. Mwenya, 2001, canvassed for the characterization and evaluation of the production characteristics. There also exist in the literature some attempts to carry out genotype profiling of indigenous goats and cattle in Zambia (Musimuko, 2014).

In an attempt to determine genetic parameters for the cattle breeds of Zambia, (Musimuko, 2014) obtained the cattle pedigree and performance records of a private farm. This is an isolated case which ought to be

encouraged and expanded through linking and sharing of data even between the public and private sectors. The Global Open Data Agricultural and Nutrition (GODAN) supports the proactive sharing of open data to make information about agriculture and nutrition available, accessible and usable to deal with the urgent challenge of ensuring world food security.

There is little information on genetic parameters for any traits of economic importance in any of the farm animals of Zambia. This is not surprising given that genetic parameters are computed from large data sets collected over a long period of time and for which information is available on the pedigree and performance. However, we can start small with whatever is available and build the data sets over time. There can be leverage on the Herdbook Society of Zambia and its members, commodity based associations and research stations to build up this livestock production database. However, successful implementation is premised on individual animal identification. This has the advantage of being unique such that an animal can be traced from farm to fork since all transactions from birth to slaughter can be captured. Management practices such as weaning, weighing, vaccination, dipping, spraying, sale, slaughter and processing are captured in the lifetime of the animal. This information can also be of value in selecting siblings or near relatives. The individual animal identification is thus the bedrock of a very good livestock data management system, even when reared as herds or flocks. Details of the sire and dam of the animal is of very high importance even if it was by use of the artificial insemination technique. It is important to stress again that data is the lifeline of animal breeders. Information on the males is of very high importance because of their wide and long lasting genetic distribution in a population.

Livestock Data Management systems

It must be emphasized that data collection is not an end in itself. It is a means to achieve a set of targets. Hence, data collected

must be inspected for correctness and accuracy. If errors are detected in the data collected, then a process of data cleaning must be employed to remove such errors. Thereafter, the data must be presented in a form that can be analyzed and a report generated. To achieve these steps, it is essential that there must be a robust Livestock data management system. The following are the current livestock management systems in use in a number of countries: FAOSTAT, WAHIS, LIMS, LIMS(2), DAD-IS, DAGRIS, ARIS 1, LITS and ARIS 2.

Currently, Zambia like other SADC countries has installed the SADC supported LIMS as the official data management system which enables the sharing of data on livestock across the SADC countries. It should be noted that FAO also have DAD-IS for reporting animal genetic resources information. The development of ARIS2 by the African Union Interafrican Bureau for animal Resources has provided another avenue for livestock data management across Africa. The advantage of ARIS2 is that it could be a stand-alone system or serve as a backup for all the data management platforms. It is also able to link and share data from the different information systems. Thus, ARIS 2 is able to harmonize data management across different platforms (Ahmed 2017).

Nevertheless, it is risky to rely on a single data management system should there be a system crash with damage caused to the data, hardware and software due to natural disasters like fires, floods, earthquakes, power failures, etc. It is therefore recommended that each data management system be kept separate but with the proviso that they should be linked and capable of sharing data on various segments of the livestock data capture chain such as health, breeding, socio-economics, slaughter and marketing.

Conclusions

Efforts should not be spared in the development of national livestock databanks for genetic improvement purposes. It is evident that countries would require technical and financial assistance from international

organizations. Countries should also be given the latitude to leverage on existing livestock data platforms.

Statement of competing interests

There was no funding received for the research. The author has no competing interests

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EFFETS DU PROTOCOLE ANESTHÉSIQUE ACÉPROMAZINE-KÉTAMINE SUR LES PARAMÈTRES VITAUX ET BIOCHIMIQUES LORS DE L'ORCHIDECTOMIE ET LA CAUDECTOMIE DES CHIENS DANS LES CLINIQUES VÉTÉRINAIRES DE YAOUNDÉ (CAMEROUN)

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Résumé

Cette étude a été réalisée dans le but d'évaluer les effets anesthésiques du protocole acépromazine-kétamine sur les paramètres vitaux et biochimiques lors de l'orchidectomie (10 sujets) et la caudectomie (7 sujets) chez les chiens dans la ville de Yaoundé (Cameroun). Tous les chiens ont été initialement prémédiqués à l'acépromazine et environ dix minutes après, l'induction anesthésique a été réalisée à la kétamine. Au cours des différents temps opératoires, des paramètres vitaux (fréquence cardiaque, fréquence respiratoire et température rectale) ont été enregistrés à des temps T_i (10 minutes avant la prémédication), T₀ (juste après l'induction), T₁ (25 minutes après le début de l'incision), T₂ (à la fin de la chirurgie) et T₃ (20 min après la fin de la chirurgie). Les concentrations sériques de cortisol (µg/dl), glucose (mg/dl), urée (mg/dl) et créatinine (mg/dl) ont été quantifiées aux temps T_i, T₀, T₁, T₂ et T₃. Les résultats indiquent que les qualités de sédation, d'induction et de réveil ainsi que les paramètres vitaux se sont avérées globalement excellents chez tous les animaux durant la prise en charge chirurgicale. Bien que procurant des effets narcotique, myorelaxant et de sécurité, ce protocole présente un inconvénient majeur : l'absence d'analgésie (cortisolémie et glycémie élevées).

Mots clés : chiens, acépromazine-kétamine, castration, caudectomie, Yaoundé.

EFFECTS OF ACEPROMAZINE-KETAMINE ANAESTHESIA ON VITAL AND BIOCHEMICAL PARAMETERS DURING ORCHIDECTOMY AND TAIL DOCKING OF DOGS IN VETERINARY CLINICS IN YAOUNDE (CAMEROON)

Abstract

This study was conducted to evaluate the anesthetic effects of the acepromazine-ketamine protocol on physiological and biochemical parameters during orchidectomy (10 animals) and tail docking (7 animals) in dogs in the city of Yaoundé (Cameroon). All dogs were initially premedicated with acepromazine and about ten minutes later ketamine was used for induction. During the various operating times, vital parameters, including the heart rate, respiratory rate and rectal temperature were recorded at times T_i (10 minutes before premedication), T₀ (just after induction), T₁ (25 minutes after the incision begins), T₂ (at the end of the surgery) and T₃ (20 minutes after the end of the surgery). Serum concentrations of cortisol (µg/dl), glucose (mg/dl), urea (mg/dl) and creatinine (mg/dl) were quantified at times T_i, T₀, T₁, T₂ and T₃. The results indicated that the quality of sedation, induction and waking and vital parameters was generally excellent in all animals during surgical management. This protocol, while providing narcotic, myorelaxant and safety effects had a major drawback: the absence of analgesia (higher cortisolemia and glycemia).

Key words: dogs, acepromazine-ketamine, castration, tail docking, Yaounde

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Introduction

La reconnaissance, la lutte et le soulagement de la douleur chirurgicale chez les animaux sont un sujet d'intérêt pour la profession vétérinaire, le grand public et la communauté de la recherche, et l'un des premiers buts de la médecine en général et vétérinaire en particulier. Dans la pratique vétérinaire pour animaux de compagnie, l'anesthésie tient une place de plus en plus importante. En effet, les cliniques vétérinaires proposent à leur clientèle d'effectuer des actes de plus en plus compliqués, tels que les chirurgies orthopédiques. Ces opérations longues et douloureuses requièrent de la part du vétérinaire une bonne maîtrise de la douleur ainsi qu'un maintien rigoureux des fonctions vitales de l'animal. La mise en œuvre d'un protocole anesthésique adapté, couplé à une surveillance continue de l'animal anesthésié apparaissent donc indispensables. Le terme « anesthésie » désigne littéralement une perte de la sensibilité. L'anesthésie générale désigne donc une perte totale de la sensibilité, alors que l'anesthésie locale désigne une perte de sensibilité localisée à une partie du corps. Mise en œuvre en quatre phases à savoir la prémédication, l'induction, l'entretien et le réveil, l'anesthésie générale est définie par une perte de conscience, une analgésie ainsi qu'une relaxation musculaire (Dugdale, 2010). L'obtention de cet état permet de réduire la réponse endocrinienne et autonome de l'animal anesthésié au stress induit par l'acte chirurgical et, de cette façon, de maintenir au mieux ses fonctions vitales (Cuvelliez *et al.*, 2007). Toutefois, il existe une variété d'autres facteurs qui peuvent affecter ces paramètres physiologiques comme le stress, l'hypovolémie et les maladies intercurrentes (Atalan *et al.*, 2002 ; Gaynor and Muir, 2008 ; Bergamasco *et al.*, 2010).

L'orchidectomie et la caudectomie canines représentent des actes chirurgicaux de convenance les plus communément pratiqués en clinique canine au Cameroun (Kouamo *et al.*, 2018). Les animaux subissant cette intervention sont généralement soumis à une anesthésie

générale pour la plupart. Le protocole utilisé par le praticien doit à la fois assurer une immobilité complète de l'animal, nécessaire au bon déroulement de la procédure, mais également une analgésie de qualité permettant de prendre en charge la douleur générée par ces actes invasifs. De plus, le protocole anesthésique doit être le moins cher possible afin que le vétérinaire libéral puisse rester compétitif sur les tarifs de l'orchidectomie et/ou de la caudectomie, et il doit pouvoir être réalisé sur un animal peu coopératif.

La kétamine hydrochloride, un analogue de la phénylcyclidine, est un anesthésique général à action brève. Elle inhibe sélectivement les zones du système nerveux central, et stimule simultanément d'autres systèmes (Thurmon *et al.*, 1975 ; Sawyer, 1998). Elle a été utilisée avec succès comme agent anesthésique général chez une variété d'animaux domestiques, incluant les chats, les chiens, les chevaux et les moutons (Stoelting, 1999 ; Hall and Clarke, 1991). L'administration d'acépromazine en combinaison avec la kétamine présente des avantages distincts. L'acépromazine réduit la dose de kétamine nécessaire pour une période donnée d'anesthésie, augmente le degré et la durée de la relaxation musculaire, empêche les mouvements réflexes des membres et prolonge le temps de réveil et de récupération complète (Thurmon *et al.*, 1973).

L'étude menée par Kouamo and Kana (2018) au Cameroun a montré que l'acépromazine et la kétamine sont très utilisées respectivement pour la prémédication et l'induction de l'anesthésie. Cette étude a également déploré une sous-utilisation des analgésiques puissants à l'instar des opioïdes lors de l'élaboration des protocoles anesthésiques. Fort de cela, il apparaît indéniable d'évaluer l'effet anesthésique procuré par l'association acépromazine-kétamine (AK) et son rôle dans la réussite de l'acte chirurgical. C'est dans cette optique que cette étude a été menée afin d'évaluer l'effet du protocole AK sur les paramètres vitaux (fréquence respiratoire (FR), fréquence cardiaque (FC), température (T0)) et métaboliques (cortisolémie, glycémie, urémie et créatininémie) lors de l'orchidectomie et la

caudectomie des chiens au Cameroun.

Matériels et méthodes

L'étude s'est déroulée au sein de diverses cliniques vétérinaires de la ville de Yaoundé. Dix-sept (17) chiens mâles de race confondue, de poids moyen 12,45Kg (11,78Kg pour l'orchidectomie et 12,17Kg pour la caudectomie) et d'âge moyen de 20,35 mois (6,28 mois pour l'orchidectomie et 14,55 mois pour la caudectomie) présentés pour une orchidectomie ou une caudectomie ont été inclus dans l'étude. Deux lots ont été constitués : un groupe de 10 animaux pour l'orchidectomie et un autre de 7 animaux pour la caudectomie.

Un examen clinique préopératoire était réalisé au préalable et pour chaque animal admis à l'étude, les paramètres physiologiques (FR, la FC et la To) étaient notés. La couleur des muqueuses oculaire et gingivale était évaluée avant de procéder au prélèvement sanguin via un cathéter intraveineux. Le sang ainsi prélevé a permis d'évaluer les paramètres biochimiques suivants : le cortisol, le glucose, l'urée et la créatinine. Les animaux peu ou pas coopératifs étaient systématiquement écartés de l'étude.

L'animal admis en salle de chirurgie était alors prémédiqué à l'acépromazine (Calmivet®) à la dose de 0,05 mg.Kg-1 en IV ou en IM puis quelques minutes après, l'induction (T0) était effectuée à la kétamine (Kétamine chlorhydrate®) à la dose 5-10 mg.Kg-1 en IV. Juste après la prémédication et l'induction, la qualité de la sédation et de l'induction était évaluée selon les critères décrits par Pottie *et al.*, (2008), les paramètres physiologiques relevés, le prélèvement sanguin effectué et la profondeur de l'anesthésie évaluée (réflexes palpébral et cornéen et la myorelaxation). Les animaux étaient alors préparés selon le type de chirurgie qu'ils devraient subir. L'orchidectomie et la caudectomie ont été réalisées selon les techniques décrites par Olson *et al.*, (2001) et MacPhail (2013), respectivement.

Vingt-cinq minutes (25 min) environ après le début de l'intervention noté T1, les paramètres étaient à nouveau notés à savoir

les paramètres physiologiques (FR, FC et T°), la qualité de la profondeur de l'anesthésie et les prélèvements de sang pour l'analyse biochimique étaient effectués. Ces observations permettaient de surveiller la profondeur de la narcose et la stabilité des fonctions vitales au cours de l'anesthésie. A la fin de la chirurgie (T2), correspondant à la réalisation du nœud du dernier point de suture et vingt minutes après la fin de la chirurgie (T3), les mêmes paramètres que précédemment évalués ont été mesurés. Le comportement, le délai de réveil (TR) et la qualité du réveil de l'animal ont été également évalués et notés sur la fiche de renseignement du patient. Après avoir relevé les paramètres de l'étude, le suivi post-opératoire était effectué par administration d'analgésique (Phenylarthrite®), antibiotique si nécessaire (Penstrep® ou Vetospray® en pansement). La figure 1 est un récapitulatif du protocole expérimental mis en œuvre pour évaluer l'efficacité du protocole anesthésique.

Les échantillons de sang prélevés dans les tubes secs étaient immédiatement centrifugés à 2500 tours pendant 10 minutes. Le sérum ainsi obtenu était conservé à -20°C pour des analyses biochimiques à l'aide du kit ELISA du cortisol (Fortress diagnostics. United kingdom) et les kits Cypress diagnostic pour le glucose, l'urée et la créatinine.

Les données ont été enregistrées et reportés sur le tableur Microsoft Excel 2016. L'analyse statistique a été fondée sur des statistiques descriptives, des analyses de Student à l'aide du programme de statistique STAGRAPHICS Centurion XVII.I. Pour les variables quantitatives ne suivant pas une distribution normale, les tests de Wilcoxon et de Kruskal-Wallis ont été utilisés pour la comparaison des différentes moyennes. Les résultats ainsi obtenus ont été présentés sous la forme de moyenne \pm EC (Ecart-type) et de pourcentage (%) au seuil de significativité de 5 %.

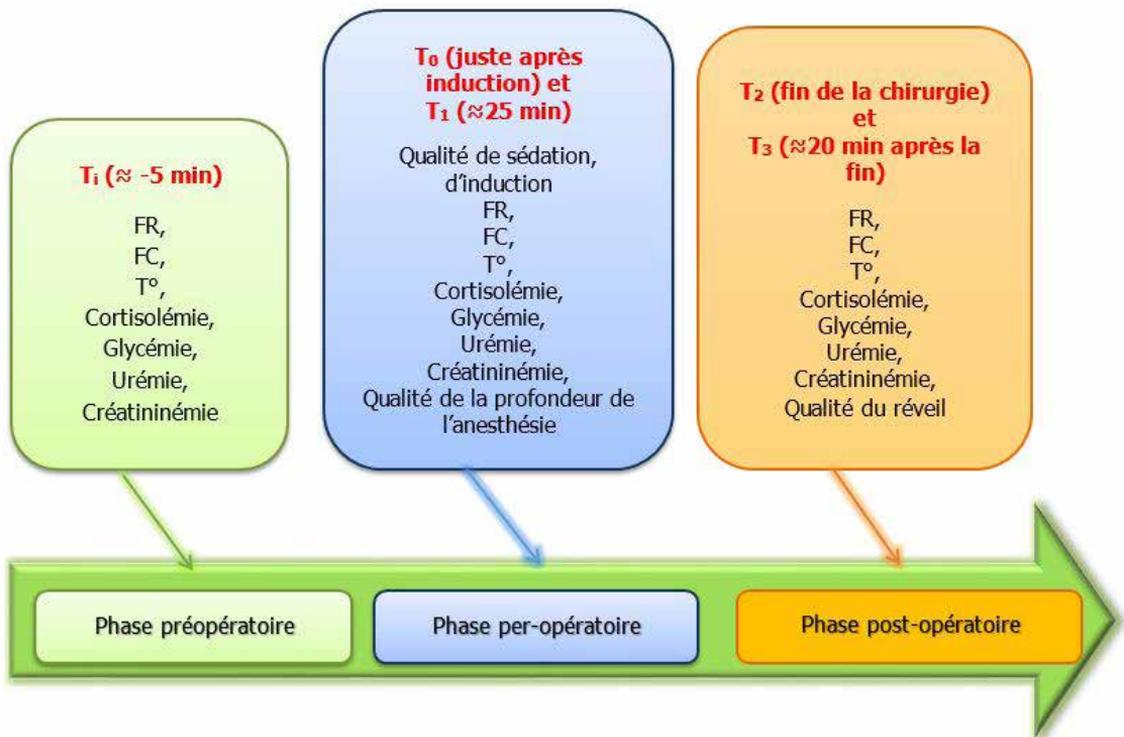


Figure 1 : Protocole expérimental mis en œuvre pour évaluer le protocole acépromazine-kétamine

Résultats

Sédation et induction

La durée moyenne du temps chirurgical variait significativement de l'orchidectomie ($46,6 \pm 5,31$ min) à la caudectomie ($54,28 \pm 3,04$). Le protocole anesthésique AK a procuré une sédation profonde et une excellente induction chez 94% des animaux contre 6% de sédation modérée et de bonne induction, respectivement. Malgré la persistance des réflexes cornéen et palpébral, la myorelaxation était de 100%.

Evolution des variables physiologiques au cours des interventions chirurgicales

Le tableau 1 représente l'effet du protocole AK sur les paramètres physiologiques au cours de l'orchidectomie et de la caudectomie. Globalement les moyennes de la FR augmentent à T_i pour chuter graduellement de T_1 à T_3 . Le protocole anesthésique n'a induit aucune différence significative sur les valeurs de la FR tout au long de l'intervention dans les deux

cas chirurgicaux : $p = 0,11$ (orchidectomie) et $p = 0,94$ (caudectomie). Concernant la FC, aucun changement significatif n'a été relevé en général ($p = 0,06$) et au cours de l'orchidectomie ($p = 0,19$) en particulier. Cependant lors de la caudectomie, la FC a significativement évolué jusqu'à 138 bpm 25min après l'induction pour chuter à 126 bpm 20min après la fin de la chirurgie ($p = 0,03$). La température quant à elle a progressivement baissé et de manière significative pour atteindre $37,82^\circ\text{C}$ et $37,77^\circ\text{C}$ lors de l'orchidectomie et la caudectomie, respectivement ($p = 0,00$).

Evolution des variables biochimiques au cours des interventions chirurgicales

Le tableau 2 compile les résultats obtenus de l'analyse biochimique sanguins. Ces résultats indiquent en terme de cortisolémie une augmentation progressive significative de façon générale ($p = 0,00$) et au cours de l'orchidectomie ($p = 0,01$) en particulier, mais non significative au cours de la caudectomie ($p = 0,35$). En effet le taux de cortisol devient

Tableau I : Effets du protocole acépromazine-kétamine sur les paramètres physiologiques

Paramètres	Types chirurgie	Temps d'anesthésie (en minutes)							p-value
		T _i	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	
Fréquence respiratoire (mvts/min)	Moyenne ± EC	32,12 ± 5,98	30,35 ± 5,8	30,70 ± 6,32	28,59 ± 6,19	27,18 ± 5,25			0,14
	Castration	31,8 ± 7,50	29,4 ± 5,25	30 ± 6,32	26,4 ± 5,8	25,2 ± 3,79			0,11
	Caudectomie	32,57 ± 3,20	31,71 ± 6,68	31,71 ± 6,68	31,71 ± 5,71	30 ± 6			0,94
	p-value	0,80	0,46	0,60	0,08	0,06			
Fréquence cardiaque (bpm)	Moyenne ± EC	118,94 ± 17,90	122,82 ± 14,71	131,18 ± 11,34	127,65 ± 10,66	120 ± 11,81			0,06
	Castration	111,6 ± 17,48	119,4 ± 17,54	126,4 ± 10,91	122,2 ± 10,36	115,8 ± 12,98			0,19
	Caudectomie	129,43 ± 13,35 ^a	127,71 ± 10,23 ^a	138 ± 8,48 ^{ab}	135,43 ± 4,72 ^{ab}	126 ± 6,93 ^b			0,03
	p-value	0,04	0,28	0,01	0,01	0,08			
Température (°C)	Moyenne ± EC	38,70 ± 0,43 ^a	38,52 ± 0,39 ^a	38,17 ± 0,46 ^b	37,98 ± 0,42 ^{bc}	37,8 ± 0,32 ^c			0,00
	Castration	38,63 ± 0,5 ^a	38,49 ± 0,42 ^a	38,13 ± 0,53 ^{ab}	37,57 ± 0,44 ^{bc}	37,82 ± 0,32 ^c			0,00
	Caudectomie	38,81 ± 0,30 ^a	38,57 ± 0,35 ^{ab}	38,23 ± 0,37 ^{bc}	38 ± 0,42 ^{cd}	37,77 ± 0,33 ^d			0,00
	p-value	0,4	0,68	0,84	0,89	0,77			

^{a,b,c} : les moyennes affectées des lettres différentes dans la même ligne sont significativement différentes (p<0,05).

Tableau 2 : Evolution des paramètres biochimiques lors de la castration et la caudectomie au cours du temps d'anesthésie.

Paramètres	Types chirurgie	Temps d'anesthésie (en minutes)						p-value
		T ₁	T ₀	T ₁	T ₂	T ₃	T ₃	
Cortisolémie	Moyenne ± EC	8,59 ± 3,22 ^a	9,64 ± 5,04 ^{a,b}	13,11 ± 6,99 ^{a,b}	15,55 ± 7,15 ^{b,c}	10,70 ± 4,54 ^c	0,00	
	Castration	7,62 ± 2,04 ^a	9,36 ± 3,94 ^{a,b}	12,44 ± 5,38 ^{b,b,c}	15,24 ± 6,53 ^{b,c}	11,16 ± 4,58 ^c	0,01	
	Caudectomie	9,96 ± 4,19	10,03 ± 6,64	14,06 ± 9,23	16 ± 8,48	10,05 ± 4,75	0,35	
	p-value	0,4	0,79	0,65	0,84	0,63		
Glycémie	Moyenne ± EC	83,67 ± 31,91 ^a	73,43 ± 24,29 ^a	123,82 ± 35,99 ^b	149,23 ± 47,45 ^b	129,20 ± 58,65 ^b	0,00	
	Castration	88,68 ± 36,50 ^a	77,84 ± 29,86 ^a	139,71 ± 36,28 ^b	165,11 ± 38,80 ^b	139,65 ± 70,46 ^b	0,00	
	Caudectomie	76,51 ± 24,80 ^a	67,13 ± 12,53 ^a	101,13 ± 21,38 ^{a,b}	126,55 ± 52,21 ^b	114,27 ± 35,86 ^b	0,00	
	p-value	0,46	0,59	0,02	0,1	0,39		
Urémie	Moyenne ± EC	38,55 ± 14,41	35,14 ± 14,5	44,99 ± 20,31	42,99 ± 21,24	36,62 ± 17,05	0,43	
	Castration	42,10 ± 13,60	41,04 ± 15,40	44,78 ± 20,37	44,17 ± 19,59	38,52 ± 16,07	0,93	
	Caudectomie	33,49 ± 15,00	26,71 ± 8,00	45,31 ± 21,85	41,30 ± 24,93	33,91 ± 19,31	0,39	
	p-value	0,24	0,04	0,96	0,79	0,6		
Créatininémie	Moyenne ± EC	1,26 ± 0,76	1,14 ± 0,79	1,44 ± 0,69	1,30 ± 0,80	1,22 ± 0,86	0,41	
	Castration	1,18 ± 0,91	1,13 ± 0,63	1,38 ± 0,71	1,36 ± 0,99	1,26 ± 1,01	0,49	
	Caudectomie	1,37 ± 0,53	1,17 ± 1,04	1,52 ± 0,70	1,21 ± 0,46	1,17 ± 0,67	0,85	
	p-value	0,14	0,92	0,73	0,81	0,88		

^{a,b,c} : les moyennes affectées des lettres différentes dans la même ligne sont significativement différentes (p<0,05).

élevé de T1 à T3 lors de l'orchidectomie. De même la cortisolémie reste élevée de T0 à T3 lors de la caudectomie. La glycémie reste élevée de façon significative de T1 à T3 en général et au cours des deux cas d'intervention ($p = 0,00$). Aucune différence significative ($p = 0,93$ lors de l'orchidectomie et $p = 0,39$ lors de la caudectomie) n'est observée avec l'urémie au cours du temps avec des valeurs restant dans l'intervalle de référence. L'évolution de la créatininémie sérique n'a montré aucune différence significative en général ($p = 0,41$) et au cours de chaque cas chirurgical ($p = 0,49$ pour l'orchidectomie et $p = 0,85$ pour la caudectomie).

Evaluation du délai et de la qualité du réveil

Le suivi post-opératoire des chiens a permis de suivre le réveil. A cet effet, le délai entre la fin de la chirurgie et le positionnement de l'animal en décubitus sternal n'était pas significatif entre l'orchidectomie ($8,8 \pm 1,03$ min) et la caudectomie ($9,28 \pm 1,50$ min). La qualité du réveil mesurée via l'échelle descriptive simple (SDS) a révélé que tous les chiens, quel que soit leur groupe (orchidectomie ou caudectomie) ont présenté un réveil «doux» et non agité.

Discussion

Au cours de cette étude, aucun animal ne présentait de complications consécutives à la prémédication à l'acépromazine, contrairement au myosis observé par Stephan *et al.*, (2003) et aux crises convulsives (Lemke, 2007) observées avec l'acépromazine utilisée seule chez les chiens. Aucun animal n'a présenté de vomissement après la prémédication ; ce qui corrobore les résultats rapportés par Valverde *et al.*, (2004) et Monterio *et al.*, (2009) qui ont observé une réduction de 45 à 18 % d'incidence de vomissement chez les chiens traités à l'acépromazine 15 min avant administration des opioïdes. La sédation profonde (94 %) observée est similaire à celle obtenue par Hofmeister *et al.*, (2010) avec l'administration d'acépromazine ou d'acépromazine-hydromorphe et par Kojima *et al.*, (1999)

par l'association acépromazine-butorphanol. De plus Nithin (2016) en Inde a rapporté que les associations acépromazine-butorphanol et acépromazine-tramadol avaient entraîné une sédation profonde respectivement à 83,33 % et 66,67 % et une sédation modérée à 16,67 % et 33,33 % chez 16 chiens ayant subi une chirurgie orthopédique. Ainsi, l'acépromazine se révèle être un bon sédatif. L'induction de l'anesthésie à base de la kétamine dans cette étude n'a pas présenté d'effets indésirables tels que les convulsions et les crises telles que rapportées par Nithin (2016) lors de l'induction combinant la kétamine-diazépam sur les chiens. Contrairement, Ranpariya *et al.*, (2013) et Henao-Guerrero and Ricco (2014) avaient observé une apnée lors de l'induction avec kétamine-midazolam et kétamine-diazépam, respectivement. La kétamine devrait être administrée en association avec un sédatif pour réduire ses effets secondaires. Dans la présente étude, l'acépromazine aurait atténué les effets secondaires de la kétamine (convulsion, vomissement, nausée) (Muir, 1995; Junot and Touzot jourde, 2015).

La présence des réflexes palpébral et cornéen n'est cependant pas un indicateur absolu d'un niveau peu profond d'anesthésie, car ces réflexes ne sont pas abolis par les agents dissociatifs contrairement aux autres agents anesthésiques comme les barbituriques et les agents volatils (White and Field, 1987 ; Booth, 1988). Durant cette étude, tous les chiens ont eu un bon niveau de relaxation musculaire pendant l'anesthésie. Cet effet serait induit par l'acépromazine qui en plus d'être un sédatif, est un myorelaxant (Thurmon, 1997; Junot and Touzot-jourde, 2015) ; ce qui a annihilé l'effet myotonique de la kétamine (Branson and Gross, 2001 ; Brainard and Hofmeister, 2012).

Les variations de la FR au cours de l'orchidectomie sont similaires à celles obtenues avec l'AK (Baniadam *et al.*, 2007), la kétamine seule (Iida *et al.*, 1997; Kamiloglu *et al.*, 2003) et l'association xylazine-kétamine (Naddaf *et al.*, 2014). Par contre, Farver *et al.*, (1986) ont montré que l'évolution de la FR était plutôt significative avec l'AK. En comparant l'orchidectomie à la caudectomie, le protocole

AK aurait plus maintenu la FR dans les valeurs usuelles lors de l'orchidectomie (28,56 mvts/min en moyenne), que lors de la caudectomie (31,54 mvts/min en moyenne); ce qui indiquerait que la caudectomie serait une intervention plus stressante que l'orchidectomie. Cependant, la kétamine est largement utilisée dans un souhait de conserver une respiration spontanée (Mion, 2001).

L'augmentation des valeurs moyennes de la FC obtenue dans les deux groupes au cours de cette étude est similaire avec celles rapportées avec la kétamine et le propofol respectivement par Thejasree *et al.*, (2017) et Kumar *et al.*, (2014) et avec la kétamine-diazépam (Beteg *et al.*, 2010). Cette tachycardie observée à T1 serait attribuable à l'effet cardio-stimulateur de la kétamine caractérisé par l'activation du système sympathique associé à la perte de conscience (Muir and Gadawski., 1998). Après T1, une chute progressive de la FC est observée dans les deux cas chirurgicaux, ce qui corrobore les observations obtenues lors de l'usage du propofol seul par Thejasree *et al.*, (2017). Hughes and Nolan (1999), Gilbert *et al.*, (2003) et Yamashita *et al.*, (2004) ont également enregistré une diminution de la FC durant l'anesthésie au propofol-fentanyl chez les chiens et ceci s'expliquait par le fait que le fentanyl augmenterait le tonus parasympathique et provoquerait une bradycardie avec les effets minimes sur la contractilité du myocarde.

Les variations de la température observées après l'administration de l'AK ont également été rapportées par Ali *et al.*, (2007) et Lopez *et al.*, (2002), respectivement. Les dérivés phénothiazines par leur effet vasodilatateur causeraient la chute de la température corporelle (Popivic *et al.*, 1972; Hall *et al.*, 2001).

L'utilisation du cortisol sérique a été reconnue comme une des méthodes les plus efficaces pour évaluer la douleur chez les animaux et les humains, et par conséquent il est important pour évaluer l'efficacité analgésique d'un protocole anesthésique. Les variations élevées de la cortisolémie durant cette étude indiqueraient que la réponse au stress douloureux était très importante. Ainsi,

il serait plus convenable que le protocole AK soit associé à un analgésique afin de diminuer la réponse au stress induit par la chirurgie tel que démontré avec la combinaison acépromazine-xylazine-kétamine lors de la laparotomie chez les chiens (Naddaf *et al.*, 2014). De plus aucune modification plasmatique du cortisol n'a été observée chez les chiens avec la xylazine-kétamine et avec la médétomidine (Ambrisko, 2002).

Les valeurs du glucose sérique ont montré une augmentation significative après administration de l'AK dans les deux cas chirurgicaux. En effet Rampariya *et al.*, (2013) ont démontré que le stress associé à l'anesthésie active l'axe pituitaire de l'hypothalamus à la production de l'adrénocorticotrophine qui stimulera par conséquent la sécrétion du glucocorticoïde et donc l'élévation de la glycémie. Cette élévation significative de la glycémie a également été démontrée au cours des études menées chez les chiens avec la kétamine seule (Kumar, 1989; Sadik *et al.*, 2012) et les valeurs relevées à différents temps étaient néanmoins comprises dans la gamme de référence. Des résultats similaires ont également été obtenus avec la kétamine-diazépam (Illera *et al.*, 2000), la médétomidine et la xylazine chez les chiens (Ambrisko, 2002; Ambrisko, 2005). Avec l'acépromazine seule, une glycémie élevée significative était observée chez les chats (Hsu and Hembrough, 1982), par contre elle était non significative chez les chiens (Ionut *et al.*, 2004). L'élévation de la glycémie observée dans cette étude concomitante à l'hypercortisolémie s'expliquerait par la douleur du stress ressenti par les animaux avec ce protocole anesthésique qui aurait des propriétés moins analgésiantes. Cela semble conforter la thèse selon laquelle si une sédation ou une anesthésie générale améliore très nettement l'immobilisation et la coopération de l'animal, c'est le traitement analgésique qui va permettre d'assurer la stabilité des paramètres hémodynamiques (Levionnois, 2015). A ce sujet, Junot and Benredouane (2007) pensent qu'il serait essentiel de ne pas associer nécessairement anesthésie à perte de conscience, mais surtout à la perte de la

sensation douloureuse, et ainsi toujours inclure une molécule analgésique dans le protocole anesthésique.

Les autres paramètres biochimiques évalués au cours de cette étude tels que l'urée et la créatinine sériques n'ont révélé aucune modification au cours des différentes interventions. Ce qui indiquerait que le protocole AK n'aurait eu aucun effet indésirable sur le fonctionnement des reins (Nithin, 2016).

Le délai maximal de réveil de 11 min observé dans les deux cas chirurgicaux signifierait que le protocole AK aurait un bon effet anesthésique qui a permis d'achever la chirurgie. Ce résultat est similaire à celui obtenu par Ghurashi *et al.*, (2009) au Soudan avec la combinaison kétamine-diazépam dont le délai de réveil était de 23 min, dû à la longue durée d'action du diazépam.

Le réveil doux et calme observé serait imputé à l'acépromazine, car avec les phénothiazines, le réveil s'effectue dans de meilleures conditions et l'incidence des vomissements diminue significativement (Junot and Touzot-Jourde, 2015). A contrario, la kétamine induit notamment une dépression sur le colliculus inférieur (noyau impliqué dans la perception acoustique) et sur le corps géniculé médial (noyau impliqué dans les voies visuelles), ce qui explique l'émergence de réaction psychique au réveil (Nelson *et al.*, 1980) et lorsqu'elle est utilisée seule avec des doses répétées, elle s'accumule et est responsable de réveils dysphoriques et ralentis (Liu *et al.*, 2006). De ce fait, l'association AK induit un réveil beaucoup plus doux et calme qu'avec la kétamine seule (Farver *et al.*, 1986).

Conclusion

Cette étude montre que l'association acépromazine-kétamine bien que procurant des effets narcotique, myorelaxant et de sécurité, possède un inconvénient à savoir l'absence d'analgésie. Ainsi, il conviendrait d'y associer des molécules analgésiques spécifiques telles les opioïdes. Par ailleurs l'analgésie préventive serait fortement recommandée, car elle permet de stabiliser l'anesthésie, d'améliorer le confort

opératoire, de contrôler la sensibilisation du système nerveux central et le développement des douleurs chroniques post-chirurgie et autorise également un réveil de meilleure qualité.

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Les auteurs déclarent ne pas avoir de liens d'intérêts.

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PREVALENCE OF BOVINE AND OVINE *Paratuberculosis* IN KERICHO COUNTY AND KONOIN SUB-COUNTY, KENYA.

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Abstract

A study was carried out in Kericho County and Konoin Sub-County, Kenya to determine the presence and prevalence of *paratuberculosis* (Johne's Disease) in cattle and sheep between February and April 2015. Faecal and serum samples were collected from 423 cattle and 403 sheep in randomly selected households. Serum samples were subjected to antibody analysis by Enzyme-linked Immunosorbent Assay (ELISA). Faecal samples were examined for acid-fast bacilli by Ziehl Neelsen (ZN) staining and cultured in Herold's Egg Yolk Medium containing Mycobactin J. Thirty-five (35) selected faecal samples were tested for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by Real Time-Polymerase Chain Reaction (qPCR). qPCR was used as a confirmatory test to the other three tests. The prevalence of *paratuberculosis* by ELISA was 2.1-2.4% and 0-0.3% in cattle and sheep respectively. By ZN staining, the prevalence ranged from 4.0-16.8% and 0.2-2.2% in cattle and sheep respectively. Isolates of MAP were obtained from 2 cattle and 1 sheep faecal samples. All the positive culture (2) and ZN staining (2), 4 out of 6 positive ELISA, 7 out of 13 negative ZN staining and 10 out of 12 ZN staining inconclusive results were confirmed positive by qPCR for MAP. There was a Pearson correlation of 0.97 between the three tests and qPCR. This study confirms the presence of MAP in cattle and sheep in Kenya and provides baseline information of the disease in the area of study. *Paratuberculosis* is a notifiable disease and there is need for a wider study to determine the prevalence in the country as a basis for instituting surveillance and control measures of the disease.

Key words: *Paratuberculosis* Prevalence Cattle Sheep Kenya

PRÉVALENCE DES PARATUBERCULOSES BOVINES ET OVINES DANS LES COMTÉS DE KERICHO ET DE KONOIN AU KENYA

Résumé

Une étude a été réalisée dans le comté de Kericho et dans le sous-comté de Konoin au Kenya, dans le but de déterminer la présence et la prévalence de la paratuberculose (maladie de Johne) chez les bovins et les ovins, entre février et avril 2015. Des échantillons fécaux et sériques ont été prélevés sur 423 bovins et 403 ovins dans des ménages sélectionnés de manière aléatoire. Les échantillons sériques ont été soumis à une analyse d'anticorps par essai d'immuno-absorption enzymatique (ELISA). Des échantillons fécaux ont été examinés pour la recherche de bacilles acido-résistants par coloration de Ziehl Neelsen (ZN) et cultivés dans un milieu de jaune d'œuf de Herold contenant de la mycobactine J. Trente-cinq (35) échantillons fécaux sélectionnés ont été examinés pour rechercher la présence de *Mycobacterium avium* sous-espèce *paratuberculosis* (MAP) par réaction en chaîne à la polymérase en temps réel (qPCR). La qPCR a été utilisée comme test de confirmation des trois autres tests. La prévalence de la paratuberculose par ELISA était de 2,1 à 2,4% et de 0 à 0,3% respectivement chez les bovins et les ovins. Par coloration au

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ZN, la prévalence variait de 4,0 à 16,8% et de 0,2 à 2,2% respectivement pour les bovins et les ovins. Des isolats de MAP ont été obtenus à partir de 2 échantillons fécaux de bovins et un (1) de mouton. Tous les résultats positifs de culture (2) et de ZN (2), 4 sur 6 ELISA positifs, 7 sur 13 négatifs et 10 sur 12 résultats non concluants de ZN ont été confirmés positifs par qPCR pour MAP. Une corrélation de Pearson de 0,97 a été identifiée entre les trois tests et la qPCR. Cette étude confirme la présence de MAP chez les bovins et les ovins au Kenya et fournit des informations de base sur la maladie dans la zone d'étude. La paratuberculose est une maladie à déclaration obligatoire, et il est nécessaire de mener une étude plus vaste pour déterminer la prévalence dans le pays comme base pour la mise en place de mesures de surveillance et de contrôle de cette maladie.

Mots-clés : paratuberculose, prévalence, bovins, ovins, Kenya

Introduction

Paratuberculosis, (Johne's Disease) is a chronic, debilitating disease, mainly of cattle and sheep, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and characterized by severe, progressive emaciation. The pathogen is transmitted through ingestion of the organism shed in milk (Streeter *et al.*, 1995) and faeces of infected animals (Kreeger, 1991). Although the disease mainly affects cattle and sheep, it has been reported in other domestic and wild pseudo-ruminants and even non-ruminants (JIC, 2010). The bacterium is also implicated in Crohn's disease, an inflammatory bowel disease of humans (El-Zataari *et al.*, 2001; Naser *et al.*, 2004). The disease causes economic losses through reduced production and trade (Garcia and Shalloo, 2015). The suspected zoonotic aspect further constitutes a public health concern (Ryan and Campbell, 2006). *Paratuberculosis* is found worldwide and whereas the disease has been well documented in Europe (Hayton, 2007) and USA (Manning and Collins, 2001; Woodbine *et al.*, 2009; Pillars *et al.*, 2009), in most African countries the disease is largely un-investigated (OIE, 2011). Recently, however, it has been reported in Uganda (Okuni *et al.*, 2011, 2013) and Tanzania (Mpenda and Buza, 2014). In Kenya, the disease is suspected mostly in the high potential areas (DVS, 2004) but no confirmation has been achieved. This study aimed to confirm the presence of the pathogen and to estimate the prevalence of the disease in cattle and sheep in Kericho District. The results provide a basis for wider studies and the formulation of appropriate surveillance and control measures.

Materials and Methods

Study area

The study area comprises Kericho County and Konoin Sub-County of Bomet County, which was jointly known as Kericho District when the 2009 census was taken (KNBS, 2010). It is located within a high potential highland area in the southern part of the Rift Valley in Kenya. Administratively, the area is divided into seven Sub Counties, namely Kipkelion East, Kipkelion West, Kericho East, Kericho West, Sigowet/Soin, Buret and Konoin (Figure 1). It has 330,903 cattle and 84,905 sheep distributed in 191,237 households (KNBS, 2010). Exotic cattle breeds such as Friesian, Ayrshire and Guernsey, and crosses between exotic and indigenous are reared for dairy purposes. Dorper is the most common sheep breed. Livestock are commonly grazed either in private paddocks or in communal pastures and forests. Breeding is mostly by use of communal bulls or rams and artificial insemination is limited to farmers who can afford the service (ASDSP, 2014).

Study Design

A sample size of 384 households at 95 percent confidence level and 0.05 degree of accuracy was obtained as described by Dohoo *et al.*, (2009). However, it was increased by 10%, to 423 households, to account for non-response. In order to ensure heterogeneity of the population, a stratified sampling design was employed to select participants in all the seven Sub Counties. A proportional allocation was done to make the sample fraction constant for each stratum. In each selected household, one cow and one sheep were randomly selected.

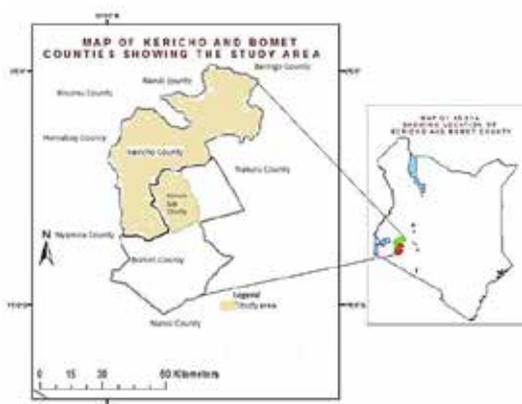


Figure 1: Map of Kericho and Bomet Counties Showing the Area of Study (Shaded)

Sample collection

Sampling was conducted between February and April 2015. The cattle were restrained in crushes whereas the sheep were manually restrained for sampling. Faecal samples weighing approximately 3gm each, were collected from the rectum of each animal and placed into labelled clean faecal pots. Blood samples were collected from the jugular veins into labelled vacutainer tubes. The faecal and blood samples were placed in a cool box and transported to the laboratory. Serum and faecal samples were stored at -20°C and 4°C respectively until use. The sampling was distributed amongst the Sub-Counties as shown in Table 1.

Laboratory analysis

ELISA

The serum samples were tested for antibodies using a commercial ELISA kit (IDEXX MAP Ab TestTM USA) according to the kit manufacturer's instructions. Briefly, all the test and control samples were diluted

1/12 in sample dilution buffer. 100 μl of serum samples from cattle and sheep, pre-incubated in a neutralizing buffer containing Mycobacterium phlei in order to avoid cross-reactions, were added to 96-well ELISA microplates which were pre-coated with a purified extract of MAP. The microplates also had wells set aside for positive and negative controls. The microplates were incubated for 45 minutes at 21°C , then rinsed 3 times with approximately 300 μl of the wash solution per well. Anti-ruminant IgG horse-radish peroxidase conjugate (100 μl), diluted 1/10 in dilution buffer, was added to each of the wells and the plates incubated for 30 minutes at 21°C . The wells were rinsed 3 times before addition of 100 μl of Tetramethyl benzidine (TMB) substrate solution. The microplates were incubated for 15 minutes at 21°C in a dark room and the reaction stopped by addition of 100 μl of "stop solution" (5N H₂SO₄) provided in the kit, to each well. The microplates were read using an ELISA Reader (Halo LED 96, Dynamica, UK) at 450nm wavelength. The test was considered valid if the mean optical density (OD) reading of the positive control on the microplate was greater than 0.350 and the ratio of the mean OD values of the positive and negative controls was greater than 3. For each sample, the Sero positivity (S/P) percentage was calculated using the following formula: $S/P\% = (\text{OD Sample} - \text{OD NC} / \text{ODPC} - \text{ODNC}) * 100$, where OD_{Sample} is the reading for the test sample; OD_{NC} is the reading for the negative control; and OD_{PC} is the reading for the positive control. S/P % of less than or equal to 60% were considered "negative"; greater than 60% but less than 70% "inconclusive", and greater than or equal to 70% "positive."

Table 1: Number of cattle and sheep sampled per Sub-County in Kericho County and Konoin Sub-County

Sub-County	Kericho East	Kericho West	Kipkelion East	Kipkelion West	Sigowet/Soin	Buret	Konoin	Total
Cattle	76	74	48	46	42	71	66	423
Sheep	69	64	48	45	42	69	66	403

Ziehl Neelsen staining

Smears from each of the faecal samples were prepared on glass slides using sterile wire loops, stained using Ziehl Neelsen (ZN) method and examined under a microscope for acid-fast bacilli (AFB). The results were recorded as either “positive” (+ve) where clumps (three or more organisms) of small (0.5–1.5µm), strongly acid-fast bacilli were observed, “Inconclusive”(+) where single AFB were observed scattered in the smear or “negative”(-ve) where no AFB were observed in the whole smear (OIE, 2011).

Faecal culture

Faecal samples were processed for MAP culture as described by Office International des Epizooties, (2012). Briefly, 2g of faecal sample were put into a 50ml-Falcon tube. Thirty five mls of de-ionised, distilled water, was added and mixed thoroughly using a vortex mixer. The mixture was allowed to settle for 30 minutes. A supernatant of 25-30mls, was then removed and centrifuged for 20 minutes at 1,700 x g. The resultant pellet was re-suspended in 0.9% HPC-BHI solution (Sigma-Aldrich, USA) and incubated at 37°C for 24 hours to allow for decontamination. Centrifugation was then carried out for 20 minutes at 1,700 x g, the supernatant discarded and the resultant pellet re-suspended in 1ml antibiotic solution containing 100µg/ml Nalidixic acid, 100µg/ml Vancomycin and 50µg/ml Amphotericin B and incubated overnight at 37°C. Two mls of each sample was inoculated in two slants of Herold's Egg Yolk Media (HEYM) (BBLTM, Hardy Diagnostics, USA), one containing 0.2mg/l Mycobactin J (ID Vet Innovative Diagnostics, France) and the other without, and incubated aerobically at 37°C for up to 52 weeks. Cultures were examined weekly for colonies. Culture was regarded as negative for MAP where there was growth in both or none of the slants, and as positive where there was growth only in the slant with Mycobactin J (OIE, 2008).

Real Time-PCR

The qPCR test was used to confirm the results obtained by the three other tests.

Thirty five (35) faecal samples for the test were selected from both animal species as follows: two ZN and culture-positive; 12 ZN- inconclusive; 13 ZN-negative; two ZN-positive and six ELISA- positive (Table 1). The samples were processed for PCR using a DNA extraction kit (ZR Faecal DNA MiniPrep™ Catalog No. D6010 by Zymo Research, USA). Briefly, about 150mg of faecal samples were lysed with 750µl lysis solution in Lysis Tubes. The tubes were transferred into a bead beater (Spex Certiprep 2000 Geno/Grinder) which was set to run at maximum speed (500) for 5 minutes. The tubes were centrifuged at 10,000 x g for 1 minute and 400µl of the supernatant was transferred through a filter in a Collection Tube before being centrifuged at 7,000 x g for 1 minute. 1,200µl of Faecal DNA Binding Buffer was added to the filtrate in the Collection Tube and 800µl of the mixture transferred to a Column in a Collection Tube before centrifuging at 10,000 x g for 1 minute. The flow-through from the Collection Tube was discarded and the step of adding 800µl of the mixture transferred to a Column in a Collection Tube and centrifuging at 10,000 x g for 1 minute repeated. 200µl of DNA Pre-Wash Buffer was added to the Column and allowed to flow into a new Collection Tube and then centrifuged at 10,000 x g for 1 minute. A further 500µl Faecal DNA Wash Buffer was added to the Column and centrifuged at 10,000 x g for 1 minute. The Column was then transferred to a clean 1.5ml micro-centrifuge tube and 100µl DNA Elution Buffer added directly to the column matrix. This was then centrifuged at 10,000 x g for 30 seconds to elute the DNA. The eluted DNA was transferred to a prepared Zymo-Spin™ IV-HRC Spin Filter in a clean 1.5ml Eppendorf tube and centrifuged at 8,000 x g for 1 minute. The filtered DNA was stored at -20°C until use.

The DNA extracts from the faecal samples were subjected to qPCR following the manufacturer's instructions of the kit (Bactotype MAP PCR Kit by Qiagen™) which contained the primer IS900, specific for MAP (Soumya *et al.*, 2009). Briefly, 17µl of the Master Mix were pipetted into each reaction tube

before 8µl of the extracted sample DNA was added. Positive and negative control samples were included with each PCR test. The reaction tubes were closed with the corresponding caps. The tubes were loaded into a PCR system (ABI 7500 Standard Real Time PCR System) for amplification. The amplification protocol consisted of 15 min of initial denaturation at 95°C; followed by 40 cycles comprising 15s at 95°C, 30s at 60°C and 35s at 72°C. Results of qPCR were interpreted according to the kit manufacturer's instructions.

Statistical analysis

The prevalence of MAP by each of the three diagnostic tests was calculated as the percentage positive cases of the total respective species of animals examined. The proportion of samples confirmed by qPCR was calculated against the other three tests (ELISA, ZN-staining and culture) and a Pearson Correlation determined.

Results

Prevalence in this study covered all the cattle and sheep respectively in the area under study as represented in the sampled animals. The prevalence was given in the form of a range so as to include the inconclusive results that were largely validated as positive by PCR. Two serum samples from cattle were discarded after they haemolysed and out of the 421 bovine serum samples examined by ELISA, eight were positive and one was inconclusive. The lower value in the range consisted of "positive" samples only and the higher value consisted of both "positive" and "inconclusive" samples. Only one of the 403 ovine samples was positive. The prevalence in all the cattle represented by the sampled ones in the area under study ranged from 2.1-2.4% (inclusive of the inconclusive results) whereas that of sheep was 0.3%.

Acid fast staining identified AFB in 71 (17 positive and 54 inconclusive) faecal samples from cattle and nine (three positive and six inconclusive) from sheep. The prevalence in cattle ranged from 4-16.8% while that in sheep

from 0.2-2.2%.

On faecal culture, three (3) isolates, two from cattle and one from sheep were recovered. The first isolate was observed at 14 weeks, the second at 23 weeks and the last one at 40 weeks post inoculation in HEYM with Mycobactin J. The rest of the cultures were discarded due to contamination, collapse of the media or lack of growth. MAP colonies were identified as small, approximately 1mm in diameter, glistening and transparent (Nielsen *et al.*, 2004). The prevalence of *paratuberculosis* by culture was 0.47% (2/423) in cattle and 0.25% (1/403) in sheep.

The results of qPCR on the 35 selected faecal samples were as shown in Figure 2. The two samples positive by ZN-staining and culture were also positive by real-time PCR; out of 12 samples inconclusive by ZN-staining, 10 (83.8%) were positive by qPCR. Of 13 samples that were negative by ZN staining, six (46.7%) were confirmed negative and the rest turned out positive by qPCR. The two ZN-positive samples were positive and four out of six (66.7%) samples positive by ELISA were also positive by PCR (Table 2). A Pearson Correlation of 0.97 was obtained between qPCR on one side and the three tests (ZN staining, ELISA and culture) on the other.

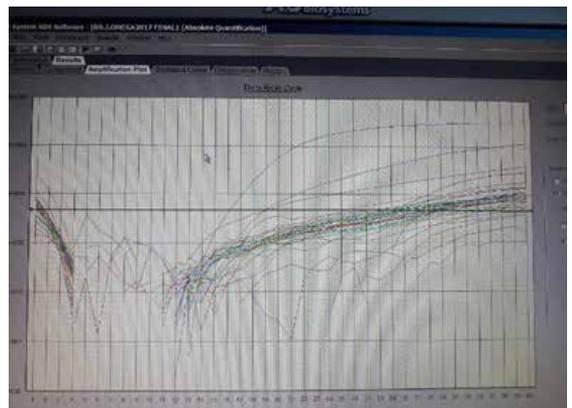


Figure 2: Results of RT-PCR for *Mycobacterium avium paratuberculosis* on selected faecal samples from cattle and sheep. The bold horizontal line is the threshold value of 35 above which the sample is considered positive.

Table 2: Comparison between Real Time-PCR and faecal culture, ZN-Staining and ELISA in diagnosis of MAP in cattle and sheep

No. of faecal samples		Results by other tests	RT-PCR	% Confirmation by RT-PCR	Pearson's Correlation	
1	1	Culture positive	2	0	100	0.97
12	0	ZN- inconclusive	10	2	83.3	
1	12	ZN-negative	7	6	46.2	
2	0	ZN-positive	2	0	100	
6	0	ELISA-positive	4	2	66.7	
22	13	-	25	10	-	

Discussion

In Kenya, reports on *paratuberculosis* in cattle have been based on clinical or post-mortem signs (Lobry, 1963), serological tests (Gossler *et al.*, 1973) and ZN staining (DVS, 2004). Paling *et al.* (1988) found antibodies to MAP in goats and camels using the complement fixation test (CFT). Reports on *paratuberculosis* in sheep are scarce (DVS, 2004). There is no previous information regarding the prevalence of the disease in any species or a definitive diagnosis (Okuni, 2013).

The prevalence of MAP by ELISA test in this investigation was 2.4% and 0.3% in cattle and sheep respectively. These results are comparable to those reported in Uganda by Okuni, *et al.*, 2011 (3%) and in Tanzania by Mpenda and Buza, 2014 (5.6%). Diagnosis of MAP by ELISA has limitations in that the test does not detect all stages of the disease, especially the earlier stages, when the pathogen is shed in the faeces, with little detectable antibody (Weber *et al.*, 2009). The earliest the test can detect the disease is one year after infection (Fletcher *et al.*, 2015). The test cannot therefore be used to support effective control strategies such as culling.

A prevalence of 4-16.8% for cattle and 0.2-2.2% for sheep was obtained by ZN staining in this study. In Egypt, Salem *et al.*, (2005) tested purposively selected samples from diseased and healthy cattle and reported a prevalence of 21% and 11% respectively. ZN-staining has low sensitivity and specificity (Weber *et al.*, 2009). In animals that tested positive by culture (85.6%

sensitivity), ZN-staining had a sensitivity of only 36.4% (Zimmer *et al.*, 1999). However, it is an important indicator of the presence of acid-fast micro-organisms in samples and works best as a screening test for Mycobacteria species in general.

Based on the principle that MAP is the only bacteria that utilises Mycobactin J in vitro for growth (OIE, 2011), culture, is considered as the gold standard for the diagnosis of MAP (OIE, 2012). However, culture is slow, laborious and expensive. Furthermore, the sensitivity is low because it may produce false-negative results due to the intermittent nature with which the MAP organisms are shed in faeces (Britton *et al.*, 2016). In this investigation, the prevalence by culture was 0.47% in cattle and 0.25% in sheep. Diagnosis of *paratuberculosis* by culture using pathological lesions in indigenous and exotic cattle has been carried out in Uganda (Okuni *et al.*, 2013). This is the first report of the isolation of MAP from cattle and sheep faecal samples in Kenya.

Real Time-PCR is highly sensitive and specific, and the ideal diagnostic test for *paratuberculosis*, although expensive. In the present study, it was used as a confirmatory test but it also detected MAP in cases that were otherwise negative or inconclusive by the other tests. The high Pearson correlation value of 0.97 obtained means that the results of the three tests collectively were highly indicative of the presence of *paratuberculosis* in the study area and that the prevalences obtained were a highly true reflection of the distribution of the disease in the cattle and sheep populations. The

validation of ZN-staining and culture of faecal, milk and water samples by qPCR has been carried out elsewhere (Ireng, *et al.*, 2009) with good results.

Conclusion

The confirmation of the presence of MAP in Kericho and Bomet Counties in particular and Kenya in general is an important development which will enable policy makers and disease control personnel to devise strategies to manage the disease and prevent further spread and losses associated with it. The prevalence of *paratuberculosis* of up to 16.8% in cattle and 2.2% in sheep provides good baseline empirical information from which further epidemiological and socioeconomic studies can be conducted.

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ASSESSMENT AND CONTROL OF TICK INFESTATION IN KALAHARI RED GOATS REARED IN HUMID TROPICS AND THE EFFICACY OF DIFFERENT ACARICIDES

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Abstract

A rainy season assessment of the tick prevalence, efficacy of two acaricides (Amitrax and an Acaricide mixture) on Kalahari Red (KR) goats reared under semi-intensive management was investigated in order to validate the strategic use of acaricides. This study was carried out at the Federal University of Agriculture Abeokuta, Nigeria from August to October 2013. The goats were identified by their tag numbers and their sex and age were recorded. The determination of the prevalence and efficacy of the two acaricides was achieved by visual observation and counting the number of ticks on the anterior surface of the right ear of the goats. Initial tick load assessments before application of the acaricides and two post treatment evaluations were carried out within a one-month interval. Data generated were analysed using SAS 9.1 using Analysis of Variance. Means were separated using Duncan's Multiple Range Test at $p < 0.05$ as a test of significance. The prevalence rate was 100%. The mean tick load was high. The post Treatment evaluation of the tick load revealed that the treatment; the age; combined factors of treatment and age; the combined factors of treatment and sex had no significant influence on the tick infestation on the goats. This result showed that the area is highly endemic for ticks. It is therefore suggested that a more frequent de-ticking programme and also the use of other acaricides for a more effective tick control on the farm be considered as a more reliable control measure.

Keywords: Kalahari Red goats, Tick load, Acaricide

ÉVALUATION ET CONTRÔLE DE L'INFESTATION DE TIQUES DANS KALAHARI ROUGE CHÈVRES ÉLEVÉS DANS LES RÉGIONS TROPICALES HUMIDES ET DE L'EFFICACITÉ DES DIFFÉRENTS ACARICIDES

Résumé

Une évaluation de la saison des pluies de la prévalence de la tique, l'efficacité des deux acaricides (mélange de Amitrax et un Acaricide) sur les chèvres de Kalahari rouge (KR) élevés en gestion semi intensive a été étudiée afin de valider l'utilisation stratégique des acaricides. Cette étude a été réalisée à l'université fédérale de l'Agriculture Abeokuta, Nigéria depuis le mois d'août à octobre 2013. Les chèvres ont été identifiés par leur numéro de balise, le sexe et l'âge a également été enregistré. La détermination de la prévalence et l'efficacité des deux acaricides a été réalisée par visual observation et compter le nombre de graduations sur la surface antérieure de l'oreille droite des chèvres respectivement. Évaluation de charge tique initiale avant l'application des acaricides et des deux post traitement évaluations ont été réalisées au sein de l'intervalle de délai d'un mois. Données générées ont été analysées à l'aide de SAS 9.1, à l'aide de l'analyse de Variance. Moyens ont été séparés selon le Test d'éventail Multiple Duncan $p < 0,05$ comme un test de signification. Le taux de prévalence était de 100 %. La charge moyenne tique était élevée. Le post évaluation du traitement de la charge de la tique a révélé que le traitement ; l'âge ; facteurs combinés de traitement et l'âge ; les facteurs combinés de traitement et le sexe a également n'eu aucune influence significative sur la ré-infestation de tiques sur les chèvres. Ce résultat indique que la zone est fortement endémique. Il est donc suggéré qu'un programme de coustil plus fréquent et aussi l'utilisation

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d'autres acaricides pour un contrôle plus efficace de la tique à la ferme considérée comme une mesure de contrôle plus fiable.

Mots-clés : Les chèvres de Kalahari rouge, charge de tiques, Acaricide

Introduction

In Nigeria, sheep and goats contribute about 10% of the livestock population, while in monetary terms, they account for about 40% of the total livestock revenue of Nigeria (McIntyre *et al.*, 1992). Small ruminants are important contributors to food production in Nigeria, providing 35% of the meat and 14% of milk consumed (Asfaw, 1997). In the humid zone of Nigeria where a mixed crop livestock production system is practiced, small ruminants account for 40% of the cash income and 19% of the household meat consumption (Zelalem and Fletcher, 1993). Owing to their high fertility, short generation interval and adaptation even in harsh environments, sheep and goats are considered as investments and insurance to provide income to purchase food during seasons of crop failure and to meet seasonal purchases such as improved seed, fertilizer and medicine for rural households.

Small ruminant production is constrained by the compound effects of diseases, poor feeding and poor management strategies according to Getachew (1995). Apart from trypanosomes and other disease agents, ticks also hinder the health and productivity of animals. Ticks are primarily blood-sucking arthropods infecting mammals, birds, reptiles and amphibians (Obadiah and Shekaro, 2012). Ticks have a major effect on the husbandry, productivity and welfare of livestock (Arends *et al.*, 1990; Uilenberg, 1995; Rehbein *et al.*, 2003).

Ticks are vectors of disease agents (such as *Babesia*, *Cowdria*, *Anaplasma*) causing anaemia, dermatitis, paralysis, as well as loss of production. Three families of ticks have been identified, but two of them are well known and of veterinary importance, hard ticks and soft ticks (Luqman *et al.*, 2007). Ticks live on and puncture, or burrow into the surface of their host's epidermis, to feed or shelter. As a result,

there may be direct damage to skin and other sub-cutaneous tissues. The presence of these ectoparasites and their salivary and faecal antigens can stimulate immune responses, in some individuals causing hypersensitivity reactions (Rehbein *et al.*, 2003). Feeding by ticks also results in significant blood loss, secondary infestation, pruritus, excoriation, alopecia and in some cases, ultimately death (Berriatua *et al.*, 2001). The behaviour of ectoparasites may also cause harm indirectly, particularly when present at high intensities, causing disturbance, increasing levels of behaviour such as rubbing, and leading to reduced time spent on grazing or ruminating and, in some cases, to self-wounding (Berriatua *et al.*, 2001). In addition, ticks act as vectors and intermediate hosts to a number of important protozoan, rickettsial, bacterial and viral diseases including zoonotic diseases which are well documented (Authur, 1970; Petney *et al.*, 2007). There is also a resultant negative implication on the total energy balance thereby resulting in decreased productivity according to Byford *et al.* (1992).

Hides and skins account for 12–16% of the total value of exports in Nigerian (Asfaw, 1997).

Ticks cause serious skin problems which result in severe economic loss to smallholder farmers, the tanning industry and the country as a whole. Bayou (1998) reported that skin problems due to external parasites cause goat skin rejections.

The reported negative impacts of ticks on husbandry, productivity and the general welfare of domestic animals and more so, the highly priced Kalahari Red goats, imported into Nigeria by the Federal University of Agriculture Abeokuta with the primary aim of upgrading the Nigerian indigenous goat breeds through crossbreeding programmes, necessitated this study. Similar works have been conducted by Raji *et al.* (1997) and Olabode *et al.* (2010) in different parts of Nigeria in different

breeds; however, there is no information on ectoparasitism in the Kalahari Red goat breed within the study area during the rainy season as this breed was only recently imported into Nigeria from South Africa. Therefore, this study was aimed at establishing the occurrence of ticks in the rainy season in the Kalahari Red goat breed herd in order to assess the possibility of the strategic use of acaricides on this breed reared in the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) Farm, Opeji, Federal University of Agriculture, Abeokuta, Nigeria.

Materials and Methods

Study site:

The study was carried out at the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) Farm, Opeji, Federal University of Agriculture Abeokuta (FUNAAB), Nigeria.

This study took place during the rainy season from August to October 2013.

Sampling:

A total of twenty-six Kalahari Red (KR) goats were used in the study. The prevalence of ticks, efficacy and durability of two different acaricides (Tartic® which contains Amitrax as the chemical composition and Acaricide mixture [Ectopour® which has Cypermethrin as the active ingredient mixed with engine oil]) were investigated using the presence of ticks and the tick load post-topical application as criteria respectively. The prevalence rate was determined by the number of goats infested with ticks and this was expressed as a percentage. The tick load assessments were carried out immediately before application of the acaricides and two times after the application (one-month intervals). The tick load was determined by counting the total number of ticks on the anterior surface of the lobbed right ear of the goats. The goats were bathed with Amitrax solution while the Acaricide mixture was applied on the back of the goats. Ticks were picked from the goats and taken to

the parasitology laboratory for identification. The sex and age of sampled animals were noted.

Statistical analysis:

Data generated were subjected to analysis of variance (ANOVA) in a General Linear Model (GLM) using SAS 9.1 for Windows statistical package. Means were separated using the New Duncan Multiple Range test of the same package at $p < 0.05$ as a level of significance.

Results

In this study, the prevalence rate of tick infestation was 100%. This means that the 26 KR goats used in this study were all infested with ticks. The species of ticks encountered were *Boophilus* and *Amblyomma* spp. The main effect of treatment on tick load was not significantly different ($p > 0.05$) in the initial and post-treatment evaluations of tick load. Table 1 shows the effect of treatment on tick load.

Table 2 shows the interactive effect of treatment and sex on the tick load. There was no significant difference ($p > 0.05$) on the tick load.

Table 3 shows the interactive effect of treatment and age of the animals on the tick load. There was no significant difference ($p > 0.05$) between the combined effects of the treatment and age of the goats with tick load.

The effect of sex on the tick load is represented in Table 4. There was significant difference ($p < 0.05$) in the tick loads between the male and female goats in the initial assessment where the males had a higher tick load than the females. However, in the 1st and 2nd post-treatment evaluations, the tick loads were not significantly different ($p > 0.05$) in both sexes.

Table 5 shows the effect of age of goats on the tick load. There was no significant ($p < 0.05$) difference on the tick load across the different age groups on tick assessment following the use of acaricides.

Table 1: Effect of acaricide types on tick load of KR goats (range values in parenthesis)

Treatment*	Tick Load		
	Initial Evaluation	1st Post Treatment Evaluation	2nd Post Treatment Evaluation
Amitrax	7.31 ± 1.35 (1-18)	28 ± 4.69 (2-55)	37 ± 6.38 (2-78)
Acaricide Mixture	18.69 ± 7.64 (1-101)	32.23 ± 8.86 (0-120)	48.92 ± 17.25 (5-205)

Table 2: Interactive effect of acaricide types and sex on tick load in KR goats (range value in parenthesis)

Treatment	Sex	Tick Load		
		Initial Evaluation	1st Post Treatment Evaluation	2nd Post Treatment Evaluation
Amitrax	Female	7.31 ± 1.35 (1-18)	28.00 ± 4.69 (2-55)	37.01 ± 6.38 (2-78)
Acaricide Mixture	Female	10.44 ± 3.83 (1-31)	39.67 ± 11.42 (9-120)	38.67 ± 16.13 (5-155)
Acaricide Mixture	Male	37.25 ± 22.46 (2-201)	15.50 ± 10.14 (0-45)	72.00 ± 45.28 (10-205)

Table 3: Interactive effects of acaricide type and age on tick load on KR goats (ranges in parenthesis)

Treatment*	Age Group* (Years)	Tick Load		
		Initial Evaluation	1st Post Treatment Evaluation	2nd Post Treatment Evaluation
Amitrax	>1 – 2	8.50 ± 1.48 (4-12)	29.83 ± 2.70 (20-37)	44.83 ± 8.44 (25-78)
Amitrax	>2 – 3	2.50 ± 0.50 (2-3)	11.50 ± 8.50 (2-20)	22.50 ± 2.05 (2-43)
Amitrax	>3 – 4	7.80 ± 2.85 (1-18)	32.40 ± 10.97 (2-55)	33.40 ± 11.22 (2-43)
Acaricide Mixture	>1 – 2	14.00 ± 7.56 (2-36)	25.50 ± 14.12 (0-65)	33.25 ± 2.37 (5-55)
Acaricide Mixture	>2 – 3	3.60 ± 0.00 (3-3)	16.00 ± 0.00 (16-16)	10.00 ± 0.00 (10-10)
Acaricide Mixture	>3 – 4	28.50 ± 15.47 (1-101)	41.17 ± 16.55 (5-120)	78.83 ± 33.51 (10-205)

Table 4: Tick load assessment of KR goats based on sex (range values in parenthesis)

Sex	Tick Load		
	Initial Evaluation	1st Post Treatment Evaluation	2nd Post Treatment Evaluation
Female	8.59 ^b ± 1.73 (1-31)	32.77 ± 5.41 (2-120)	37.68 ± 7.57 (2-155)
Male	37.25 ^a ± 22.46 (2-101)	15.50 ± 10.14 (0-95)	72.00 ± 45.28 (10-205)

^{ab}Mean with different superscripts along the column is significantly different ($p < 0.05$)

Table 5: Tick load of KR goats based on age (range values in parenthesis)

Sex	Tick Load		
	Initial Evaluation	1st Evaluation	2nd Evaluation
>1 – 2	10.70 ± 3.02 (2-30)	28.10 ± 5.43 (0-65)	40.20 ± 6.9 (5-78)
>2 – 3	2.67 ± 0.33 (2-3)	13.00 ± 5.13 (3-20)	18.33 ± 12.56 (2-43)
>3 – 4	19.09 ± 8.79 (1-101)	37.18 ± 9.93 (2-120)	58.18 ± 19.50 (5-205)

Discussion

The overall prevalence of tick infestation in the goats in this study was 100%. This shows that the environment is highly endemic for ticks. The non-significant effect of treatment on the tick load might be due to the very high population of the ticks on the vegetation (sown paddocks and the surrounding natural pastures). There was no effect of age on tick load, although Oduguwa *et al.* (2013) and Iposu *et al.* (2014) reported a higher prevalence rate in the older animals than in the younger ones. The combined effects of treatment and age on the tick load during the assessments was not significantly different ($p > 0.05$). It is possible that if a reduced number of days post application/treatment was adopted for assessment/re-evaluation, the effect of treatment would have been significant. According to earlier reports, the magnitude of losses due to tick infestation varies with genotype of host (Magona *et al.*, 2011), the species of infesting ticks (Wang *et al.*, 2007) and level of infestation (Stachurski and Lancelot, 2006). Moreover, within a genotype, losses per tick unit increase with the number of attached ticks.

Conclusion

The prevalence rate of 100% observed in this study and the high numbers of ticks in individual goats is a cause for concern whose control will require strategic applications of acaricides. Two applications per month of the acaricide mixture in the study will be sufficient to effectively control tick infestations and tick-borne diseases in goat herds. Tartic® and Ectopour® were used commonly on the farm without a desired result but the mixture Ectopour® with spent engine oil made the cypermethrin preparation to be more effective.

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EFFECT OF OIL EXTRACTION METHODS AND ENZYME SUPPLEMENTATION ON METABOLISABLE ENERGY VALUES OF SUNFLOWER SEED MEAL FOR GROWING TURKEYS

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Abstract

Metabolisable energy values of sunflower seed meal (SFM) subjected to 3 oil-extraction methods (no extraction/full fat, screw-pressed, and solvent extract method), and supplemented with or without commercial enzyme were estimated in a gavage study using forty eight male, 10-wk-old BUT turkeys (average weight of 5 kg). There were 6 treatments arranged in a 3×2 factorial arrangements of 3 oil-extraction methods supplemented with or without enzyme. Each treatment consisting of 8 turkeys housed individually in separate cages. An additional 8 turkeys were used for the assessment of endogenous losses. The main effect of processing revealed that screw-pressed SFM had the least ($P < 0.05$) AME, AMEn, TME and TMEn values. Solvent-extraction of SFM also showed improved metabolisable energy values. Enzyme supplementation of solvent-extracted SFM resulted in significant improvement ($P < 0.05$) in metabolisable energy values when compared with non-supplemented solvent-extracted SFM. Enzyme supplementation of screw-pressed SFM showed no improvement in metabolisable energy values. In conclusion, the inclusion of solvent-extracted and enzyme-supplemented sunflower meal is recommended for improved metabolizable energy values for growing turkeys.

Keywords: Enzyme supplementation, growing turkeys, metabolisable energy, oil extraction methods, sunflower meal

EFFET DES MÉTHODES D'EXTRACTION D'HUILE ET DE SUPPLÉMENTS ENZYMATIQUES SUR LES VALEURS D'ÉNERGIE MÉTABOLISABLES DES TOURTEAUX DE TOURNESOL POUR LES DINDONS EN CROISSANCE

Résumé

Les valeurs énergétiques métabolisables de tourteau de tournesol (SFM) soumises à 3 méthodes d'extraction d'huile (aucune extraction/graisse totale, à vis et méthode d'extraction par solvant) et complétées avec ou sans enzyme commerciale ont été soumises à une estimation dans une étude de gavage utilisant quarante-huit dindons BUT âgés de 10 semaines (poids moyen de 5 kg). L'étude comportait 6 traitements organisés selon des dispositifs factoriels 3×2 de 3 méthodes d'extraction d'huile complétées avec enzyme ou sans enzyme. Chaque traitement consistait en 8 dindes logées individuellement dans des cages séparées. Huit dindes supplémentaires ont été utilisées pour évaluer les pertes endogènes. L'effet principal du traitement a révélé que le SFM pressé à vis avait les plus faibles ($P < 0,05$) valeurs AME, AMEn, TME et TMEn. L'extraction de SFM au solvant a également montré une amélioration des valeurs d'énergie métabolisable. La supplémentation enzymatique de SFM soumis à une extraction par solvant a entraîné une amélioration significative ($P < 0,05$) des valeurs d'énergie métabolisable par rapport aux SFM soumis à une extraction par solvant sans supplément. La supplémentation enzymatique de SFM pressé à vis n'a montré aucune amélioration des valeurs d'énergie métabolisable. En conclusion, l'inclusion de tourteaux de tournesol extraits au solvant et enrichie d'enzymes est recommandée pour l'amélioration des valeurs énergétiques métabolisables des dindons en croissance.

Mots-clés : supplémentation enzymatique, dindes en croissance, énergie métabolisable, méthodes d'extraction d'huile, tourteau de tournesol

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Introduction

Sunflower (*Helianthus annuus*) seed meal (SFM) has been identified as a potential plant protein source that can partially or totally replace soybean meal (SBM) in composite feed for poultry. The high crude protein (280 – 420 g/kg), high methionine levels coupled with the absence of anti-nutrients in SFM makes it as a better alternative to SBM (Fafiolu *et al.*, 2012). Mechanical processing of sunflower seed is commonly employed in developing countries due to the huge cost associated with efficient oil extraction of the seed (Ravindran and Blair, 1992). The utilisation of such processed SFM has been reported to yield poor nutrient digestibility and low metabolisable energy (ME) values in poultry (Janssen and Carre', 1985).

Enzyme supplementation has been advocated for improved utilisation of processed SFM in poultry nutrition (Senkoylu and Dale, 1999). Meanwhile, conflicting findings were reported in the literature following enzyme supplementation of SFM-based diets for poultry. Improved growth (Kocher *et al.*, 2000) and nutrient digestibility (Olivera *et al.*, 2007) were reported in birds fed enzyme-supplemented SFM while Mushtaq *et al.* (2006) concluded that enzyme supplementation had no remarkable effect in diets containing SFM. Research studies on the effect of oil extraction methods on ME of sunflower meal for growing turkeys are rare. This study therefore sought to investigate the effect of oil extraction methods and enzyme supplementation on metabolizable energy values of sunflower meal for growing turkeys.

Materials and Methods

Processing and chemical analysis of sunflower seed

Dried sunflower seeds obtained from the College of Plant Science, Federal University of Agriculture, Abeokuta farm were roasted in a regulated oven (80°C for 15 min) and ground through a 2.5 mm screen to yield SFM. The SFM obtained was divided into 3 equal batches, with each batch subjected to either of the following extraction methods: manual

screw-press (placed under a hydraulic screw-press for 12 hours), solvent extraction using a Soxhlet apparatus, or no extraction (full fat). A sample of each of the final products (test ingredients) was assayed for proximate constituents (AOAC, 1995). Crude protein was determined (as N x 6.25). For mineral analysis, samples were ground, ashed at 450 °C, and digested in 10 ml of 1 mol/l HCl. The phosphorus content was determined as per AOAC Method 7.076 (AOAC 1995). Calcium was measured in the ash solution by atomic absorption spectroscopy (Techtron model AA-10, Mulgrave, VIC, Australia) at 422.7 nm. Manganese, zinc and copper were determined by atomic absorption spectroscopy using single-element hollow cathode lamps and an air-acetylene flame at 279.5, 213.9 and 248.3 nm, respectively (AOAC 1990).

Experimental management and design

A total of forty eight, 10-wk-old, British United grower turkeys (average weight of 5 kg) were allotted into 6 dietary treatments of 8 birds per treatment. The turkeys were housed individually in metabolic cages and deprived of feed for 48 h to empty their guts. The birds were given unlimited access to drinking water during the feed deprivation period. Following the expiration of this period, the turkeys were orally gavaged with 30 g of the respective SFM. Turkeys were allotted to six (6) dietary treatments in a 3 × 2 factorial arrangement of 3 processed sunflower seed meal (full-fat, screw-pressed, or solvent extraction) supplemented with or without 200 mg/kg of enzyme. The enzyme used in this study is a commercial blend of multi-enzymes consisting of endo – 1, 4 – β – xylanase (EC 3.2.1.8), endo – 1, 3 (4) – β – glucanase (EC 3.2.1.6) and endo – 1, 4 – β – glucanase (EC 3.2.1.4) produced by *Trichoderma reesei*. An additional group of eight grower turkeys was used for the measurement of endogenous losses. These birds were also deprived of feed but had access to drinking water. Following the feed-deprivation period, the turkeys meant for the assessment of endogenous losses were each offered 50 ml of glucose solution by gavage following standard

procedures (McNab and Blair, 1988). For all the groups of birds, excreta collection was done during a 48 h period after feed deprivation. The total excreta collected per bird were dried (at 60°C until constant weight) and subjected to gross energy determination using a bomb calorimeter (Adiabatic bomb calorimeter, Parr Instrument Company, Moline, IL, USA). The apparent metabolisable energy (AME), apparent metabolisable energy corrected for nitrogen (AMEn), true metabolisable energy (TME), and true metabolisable energy corrected for nitrogen (TMEn) were computed using the equations as described by Sibbald (1989).

Statistical analysis

For estimation of ME values, excreta samples collected from each turkey ($n = 8$ per treatment) served as the experimental unit. Data obtained from this study were arranged in a 3×2 factorial arrangement of 3 processed sunflower seed meals (full-fat, screw-pressed, or solvent extraction) supplemented with or without 200 mg/kg enzyme. Analysis was

done using SAS (1999) to separate the main and interaction effects. Significant means were separated using Tukey's Test. A probability of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

The determined proximate composition, fibre fraction and mineral composition of processed sunflower meal is shown in Table 1. An important aspect of feed resources is adequate information of its nutritional potential as such information will go a long way at mitigating high feed costs in raising meat type poultry (Bolarinwa and Adeola, 2012). The chemical and nutritive compositions of differently processed SFM evaluated in this study are similar to those reported by Fafiolu *et al.* (2012). Screw pressed SFM in this study contained high amounts of phosphorus possibly because of the efficiency of the oil extraction method. It should also be noted that phosphorus in the solvent extracted group

Table 1: Chemical composition and nutrient content of processed sunflower meal (SFM)

Composition (g/kg)	Full fat SFM	Solvent extract SFM	Screw-pressed SFM
Dry matter	930.50	950.00	925.00
Crude protein (N \times 6.25)	210.00	221.00	209.00
Crude extract	337.00	335.00	337.00
Crude fiber	201.00	210.00	209.00
Crude ash	50.00	51.00	50.00
Soluble carbohydrate	202.00	183.00	150.00
Fiber fractions			
NDF	332.00	310.00	330.00
ADF	112.00	97.00	117.00
Mineral Composition			
K	6.77	6.97	6.71
Ca	3.48	3.49	3.46
P	8.40	6.41	8.00
Mg	0.77	0.78	0.71
Na	0.045	0.045	0.046
Zn	269.00	269.00	269.45
Mn	251.00	251.00	253.41
Fe	128.00	128.10	128.40

was lower perhaps as a result of the efficiency of the extraction method used. It has been reported that screw pressed oils contain very small amounts of phospholipids than extracted oils (Gunstone, *et al.*, 1994).

The effect of oil extraction methods and exogenous enzyme supplementation on metabolisable energy values of SFM for growing turkeys is shown in Table 2. The main effect of processing revealed that screw-pressed SFM had the least ($P < 0.05$) AME, AMEn, TME and TMEn values. Enzyme supplementation of solvent-extracted SFM resulted in a significant improvement ($P < 0.05$) in their metabolisable energy values when compared with energy values of non-supplemented solvent-extracted SFM. The high metabolisable energy values of full-fat SFM obtained in this study agreed with previous studies which

reported high metabolisable energy values of full-fat sunflower meal for poultry (Selvaraj and Purushothaman, 2004). This has been linked with increased fat availability of the seed (Sibbald and Kramer, 1978). Past works also showed that the metabolisable energy content of full-fat sunflower meals differs widely based on the ether extract content of the sunflower seed (Daghir *et al.*, 1980). Solvent-extraction of SFM also showed improved metabolisable energy values in the present study. This could be linked with the efficient oil extraction method employed. Efficient oil extraction processes used in European countries for oil seed has been reported to improve metabolisable energy values of ingredients (San Juan and Villamide, 2001). Of all the various oil extraction methods used in the current study, screw-pressing had the least metabolisable

Table 2: The effect of oil extraction methods and exogenous enzyme supplementation on metabolisable energy values of SFM for growing turkeys

Attributes		AME	AMEn	TME	TMEn
Processing methods	Enzyme				
Full-fat		10.55 ^a	10.38 ^a	10.99 ^a	10.81 ^a
Solvent-extraction		10.69 ^a	10.52 ^a	11.14 ^a	10.97 ^a
Screw-press		7.93 ^b	7.76 ^b	8.58 ^b	8.45 ^b
SEM		1.22	1.05	1.42	1.27
	No	9.45	9.27	9.96	9.81
	Yes	9.99	9.82	10.51	10.34
	SEM	0.20	0.19	0.28	0.28
Full-fat	No	10.58 ^{ab}	10.39 ^{ab}	11.01 ^{ab}	10.84 ^{ab}
Full-fat	Yes	10.53 ^{ab}	10.36 ^{ab}	10.97 ^{ab}	10.79 ^{ab}
Solvent-extraction	No	10.13 ^b	9.96 ^b	10.59 ^b	10.42 ^b
Solvent-extraction	Yes	11.26 ^a	11.07 ^a	11.70 ^a	11.52 ^a
Screw-pressed	No	7.64 ^c	7.48 ^c	8.29 ^c	8.19 ^c
Screw-pressed	Yes	8.21 ^c	8.04 ^c	8.87 ^c	8.71 ^c
SEM		1.04	1.02	1.10	1.24
P-values					
Processing methods		<0.0001	<0.0001	<0.0001	<0.0001
Enzyme		0.150	0.073	0.099	0.077
Processing methods × Enzyme		0.025	0.021	0.028	0.030

^{a, b, c} Means on the same column with different superscript differs significantly ($P < 0.05$) AME= Apparent metabolisable energy, AMEn= apparent metabolisable energy corrected for nitrogen, TME= True metabolisable energy, TMEn= True metabolisable energy corrected for nitrogen

energy values. This could be due to the inefficient oil extraction method. However, the poor metabolisable energy content recorded in the current study with screw-pressed SFM can be attributed to the inefficient oil-extraction methods employed.

Few studies exist on the effect of enzyme supplementation on energy metabolizability of sunflower meal. A significant improvement in AME of SFM from 6.1 to 6.5 MJ/Kg by poultry birds through enzyme supplementation was previously reported (Mandal *et al.*, 2005). Smith (1968) reported that supplementation of sunflower-based diet with enzyme increased nutrient utilization of SFM in layers and broilers. Enzyme supplementation of SFM was reported to improve significantly the non-starch polysaccharides (NSP) and protein digestion in the ileum (Kocher *et al.*, 2000). Although full fat and screw-pressed SFM showed no improvement following the addition of enzyme in the present study, enzyme supplementation significantly improved the metabolisable energy values of solvent-extracted SFM. This agreed with previous findings which reported improved AME of SFM following supplementation of enzyme (Mandal *et al.*, 2005). It also agreed with previous findings which reported that problems associated with SFM could be ameliorated using multi-enzymes (Cmiljanic *et al.*, 2007; Nian *et al.*, 2011).

The metabolisable energy values of SFM obtained in this study was in line with the range reported for poultry in previous literatures (Devegowda *et al.*, 1986; Reddy, 1993). However, apparent metabolisable energy values obtained in the present study was lower than values of 18.71 and 17.05 MJ/Kg obtained for broilers by Chevalsarakul and Tangtaweewipat (1991) and Rodríguez *et al.* (1998), respectively. The variations in the findings of the present study with aforementioned authors could be due to differences in poultry species, age of bird and processing methods of sunflower meal used. AME values of oil seed meal have been reported to vary depending on many factors which include species, age (Fisher and Shannon, 1973) and processing methods (Sibbald and Slinger, 1963). Variations in the AME values of

rape seed meal have been reported following different processing methods (Sell, 1966).

In conclusion, the present study elucidated the poor energy potential of screw-pressed sunflower meal and the prospects of enzyme-supplemented solvent-extracted sunflower meal for growing turkeys. Inclusion of solvent-extracted and enzyme-supplemented sunflower meal is therefore recommended for improved metabolizable energy values for growing turkeys.

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PRIORITISATION AND CONTROL OPTIONS FOR TRANSBOUNDARY ANIMAL DISEASES (TADS) AND ZONOSSES IN THE EAST AFRICAN COMMUNITY (EAC)

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Abstract

The East African Community (EAC) currently comprises six Partner States namely: Burundi, Kenya, Rwanda, South Sudan, Tanzania, and Uganda with a human population of about 192 million. Agriculture is the mainstay of the region's economy and social development and has been the focus of important regional initiatives. Livestock Agriculture is a significant livelihood and food security enterprise among residents of the East African Community and is practiced by about 80% of the region's population. With the current stock numbers estimated at 56.6 m cattle, 61.9 m goats and 32.3m sheep, 7.9 m pigs 148.6 m poultry and 3.4 m camels, the region is among the top livestock hubs in Africa. The EAC region is also the main hub of protected areas for wild animals on the continent. That notwithstanding, the region is equally affected by animal diseases that significantly compromise production, productivity, access to market and public health. In order to design more effective disease control programmes, disease ranking and prioritisation should be carried out from time to time. The present exercise was undertaken to fulfill this objective and the methodology for the prioritisation of Transboundary animal diseases (TADs) and zoonoses in the EAC region was based on the study conducted on behalf of OIE by PHYLLUM on "Listing and Categorisation of Priority Animal Diseases, including those Transmissible to Humans. All Disease information was captured in a computerised model (Excel Sheet) that contains formulae and other linkages to help with computations. Data from the characterisation of the diseases was transferred to an iterative table where automated computation produced a weighted list of priority diseases. Both qualitative and quantitative elements of prioritisation were employed for the different diseases and for the three disease categories, namely: absent diseases, present diseases with known epidemiological status and diseases with unreliable local epidemiological knowledge. Corresponding control strategies that should be developed and implemented among other possibilities was also generated for each disease. The output from this tool was subjected to a comparative and interpretative process involving interdisciplinary experts and local decision makers, and integrating the different components of local geopolitical and socio-cultural orientation. This disease prioritisation exercise has created a clear opportunity for the EAC region to adopt measures for the systematic control and progressive eradication of animal diseases on the basis of evidence of their importance at both country and regional levels.

OPTIONS DE PRIORISATION ET DE CONTRÔLE DES MALADIES ANIMALES TRANSFRONTIÈRES (TADS) ET DES ZONOSSES DANS LA COMMUNAUTÉ DE L'AFRIQUE DE L'EST (CAE)

Résumé

La Communauté de l'Afrique de l'Est (EAC) regroupe actuellement six États partenaires, à savoir le Burundi, le Kenya, le Rwanda, le Sud-Soudan, la Tanzanie et l'Ouganda, et compte une population d'environ 192 millions d'habitants. L'agriculture est le pilier de l'économie et du développement social de la région, et a été au centre d'importantes initiatives régionales. L'élevage est une activité importante de subsistance et de sécurité alimentaire pour les résidents de la Communauté de l'Afrique de l'Est, et est pratiqué par près de 80 % de la population de la région. Abritant des effectifs animaliers actuellement estimés à 56,6 millions de bovins, 61,9 millions de chèvres et 32,3 millions de moutons, 7,9 millions de porcs, 148,6 millions de volailles et 3,4 millions de chameaux, la région est l'un des principaux pôles d'élevage en Afrique. La région de l'EAC est également le centre des aires protégées pour les animaux sauvages sur le continent. Malgré cela, la région est tout aussi affectée par des maladies animales qui compromettent considérablement la production, la productivité, l'accès au marché et la santé publique. Afin de concevoir des programmes plus efficaces de contrôle des maladies, un classement des maladies par ordre de priorité devrait se faire de temps à autre. Le processus en cours a été lancé pour atteindre cet objectif, et la méthodologie de priorisation des maladies animales transfrontalières (MAT) et des zoonoses dans la région de la Communauté de l'Afrique de l'Est (EAC) s'est basée sur les résultats de l'étude réalisée par PHYLLUM pour le compte de l'OIE sur le « Recensement et catégorisation des maladies animales prioritaires dont celles transmissibles à l'homme ». Toutes les informations sur les maladies ont été saisies dans un modèle informatisé (feuille de calcul Excel) contenant des formules et d'autres liens pour faciliter

les calculs. Les données résultant de la caractérisation des maladies ont été transférées dans un tableau itératif où le calcul automatisé a produit une liste pondérée des maladies prioritaires. Des éléments qualitatifs et quantitatifs de définition des priorités ont été utilisés pour les différentes maladies et pour les trois catégories de maladies : « maladies absentes », « maladies présentes au statut épidémiologique connu », et « maladies dont les connaissances épidémiologiques locales ne sont pas fiables ». Des stratégies de lutte correspondantes qui devraient être élaborées et mises en œuvre, entre autres possibilités, ont également été développées pour chaque maladie. Les résultats de cet outil ont fait l'objet d'un processus comparatif et interprétatif faisant appel à des experts interdisciplinaires et aux décideurs locaux, et intégrant les différentes composantes de l'orientation géopolitique et socio-culturelle locale. Ce processus de priorisation des maladies a clairement donné à la région de l'EAC l'occasion d'adopter des mesures de contrôle systématique et d'éradication progressive des maladies animales, fondées sur des données scientifiques de leur importance tant au niveau national que régional.

Introduction

The East African Community is one of the 8 Regional Economic Communities of the African Union and currently comprises 6 countries (Partner States) namely: Burundi, Kenya, Rwanda, South Sudan, Tanzania, and Uganda. The region has a total surface area of 2,462 thousand square Km and is bordered by Sudan to the north, Central African Republic and the Democratic Republic of Congo (DRC) on the west, Ethiopia, Somalia and the Indian Ocean to the East and Malawi, and Zambia to the south. The human population is estimated at 192 million. Agriculture is the mainstay of the region's economy and social development, and has been the focus of important regional initiatives. Livestock Agriculture is a significant livelihood and food security enterprise among residents of the East African Community and is practiced by about 80% of the region's population especially, for people living in the drier arid and semi-arid lands (ASALs) of the region. With the current stock numbers estimated at 56.6 m cattle, 61.9 m goats and 32.3m sheep, 7.9 m pigs, 148.6 m poultry and 3.4 m camels (1), the region is among the top livestock hubs in Africa. The region is also the main hub of protected areas for wild animals on the continent. That notwithstanding, the region is afflicted by animal diseases that significantly compromise production, productivity, access to markets and public health (2). A large number of transboundary animal diseases (TADs) and zoonoses are present in the region, with the majority being endemic. These include: Rift Valley Fever, Foot and Mouth Disease (6 serotypes), Newcastle Disease, Contagious Bovine Pleuropneumonia

(CBPP), Contagious Caprine Pleuropneumonia (CCPP), Trypanosomiasis (animal and human) Peste des petits Ruminants (PPR), Lumpy Skin Disease (LSD), Rabies, African Swine Fever, Brucellosis, Bovine/Zoonotic Tuberculosis, Sheep and Goat pox, camel pox and infectious bursal disease (Gumboro) among others (2). Other public health diseases with significant emerging importance in the region include viral haemorrhagic diseases that have been responsible for occasional outbreaks and the camel associated Middle East respiratory syndrome (MERS) caused by the Middle East respiratory syndrome coronavirus (MERS-Cov) that has been at the center of serious human mortalities in the Middle East and some South Asian Countries (3).

Consequently, the regional block has taken bold measures to improve animal agriculture by the control of animal diseases, especially the transboundary ones and those transmissible to humans and enhancing trade in animal and animal products. The East African Community (EAC) developed a strategy for the prevention and control of Transboundary Animal Diseases (TADs) and Zoonoses (4) to safeguard animal health and production, as well as public health. The strategy is a tool for implementing regional mandates emanating from the provisions of the Treaty for the establishment of the EAC. Article 5 (5) of this treaty, which sets out the objectives of the Community, stipulates that "the Community shall ensure the strengthening and consolidation of co-operation in agreed fields that would lead to equitable economic development within Partner States and which would in turn, raise the standards of living and improve the quality of life of their populations". Chapter 18 of the

Treaty specifically provides for cooperation in addressing economic and development issues including agriculture, food security, livestock multiplication and distribution, and trade among others and specifically outlines measures for the prevention and control of plant and animal diseases in Article 108. The vision of the regional TADs and Zoonotic diseases strategy is: “Wealth created through a harmonised, improved, competitive and safe integrated, market-driven sustainable livestock productivity and tourism”. The strategy aims at safeguarding human and animal health and livelihoods of the farming communities from outbreaks of TADs through building capacities in the Partner States for effective rapid detection and response to outbreaks. The harmonised strategy guarantees an effective, safe, and enhanced production and trade environment supported by capable, credible regional and Partner States’ institutional and human capacities. In addition to safeguarding human health, this capacity would enable and guarantee that the region meets the sanitary requirements of the World Organisation for Animal Health (OIE) and the World Trade Organization (WTO). The regional strategy espouses the One Health Approach to ensure effective engagement and collaboration among all relevant stakeholders (6)

Although the region has been working towards the free movement of goods and services for close to 2 decades, trade in livestock and livestock products is often rocked by bans as a result of transboundary animal diseases and other Sanitary and Phyto-Sanitary (SPS) related concerns.

To turn this situation around requires a strong focus on the management of TADs at both Partner States and regional levels. Furthermore, to attract the required attention and resources, the need to demonstrate impact and a clear prioritisation of diseases cannot be over-emphasised.

The control of animal diseases including those transmissible to humans is very weak in most of Africa, including the EAC region. This is in spite of the region having a very high burden of transboundary animal diseases and zoonoses

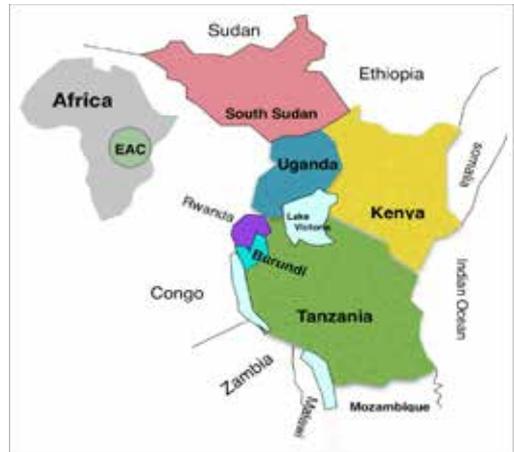


Figure 1: Map of East African Community Countries (Republic of Burundi, Republic of Kenya, Republic of Rwanda, Republic of South Sudan, United Republic of Tanzania and Republic of Uganda (Source, Google, 2016)

with a number of the 117 OIE-listed animal diseases, infections and infestations being endemic on the continent. Among the main factors responsible for this scenario are weak public health and animal health systems, weak disease control infrastructure and perennial shortages of key resources (human and financial resources), lack of public participation/ social support, poor access to the right tools and technologies among others. Further, no scientific tool has been available to guide disease control programming in the region, necessitating the identification and adoption of one with appropriate capacity building. The Phylum Tool (5) for the prioritisation and categorisation of animal diseases, including those transmissible to humans was adopted for the EAC region. Adoption of the tool will serve many important functions. These include identifying and ranking the most important diseases that warrant investment of scarce resources, bringing multi-sectoral stakeholders and experts together to build consensus on the roles and responsibilities of different players, identifying the means to deal with them, identifying and deliberating potential strategies and interventions and identification of possible policies to be developed/implemented among others.

By facilitating these processes, disease prioritisation demonstrates that the burden of disease control doesn't solely lie with the national veterinary services but is the collective responsibility of all stakeholders in the animal resources sector. Further, it demonstrates that not all diseases must be controlled using public resources, and that there are those that can be sufficiently managed by private actors in the sector. The clear identification of priorities, roles of stakeholders and the means required to control animal diseases, will catalyse more effective, efficient and sustainable processes for their prevention and control (7)

Materials and Methods

The methodology applied in the prioritisation of TADs and zoonoses in the EAC region was based on the study conducted on behalf of OIE by PHYLUM on "Listing and Categorisation of Priority Animal Diseases, including those Transmissible to Humans" (8).

The methodology involved two sequential steps in the analysis of a disease, namely; the global characterisation to assess the inherent (scientific) aspects of the disease independently of any particular local context and the local approach which aimed at assessing the disease within the specific context of the country or region in question. The global characterisation concerns itself with the intrinsic analysis of the disease based on available scientific knowledge. Its main objectives are to establish the characteristics for the description of the disease profile and its potential impact in terms of epidemiology, economic consequences and human health issues, to create consensus among stakeholders on the fundamental data on which to base the detailed analyses and identify possible gaps in terms of disease knowledge. Key aspects of the global characterisation include the presence or absence of the disease, its nature and modes of transmission. The possible control measures of the disease are then assessed to obtain an overview of the availability, effectiveness and efficiency of the tools (means) to control the disease in the event of an outbreak. The local

approach on the other hand is concerned with applying the global characterisation to the specific context of a given country or region. The impact of a disease in a territory is highly dependent on local perceptions, geography, production and trade systems and socio-cultural background among others. Thus, it is possible to prioritise the same disease differently depending on the specific contexts at the local level. The local approach is a data intensive exercise requiring an accurate overview of the production systems, the global importance of animal agriculture and the respective importance of the different categories of animals and animal products for the local economy. The key elements of the local assessment include the epidemiology of the disease, absence/presence and risk of introduction, impact, control strategies and their local feasibility and the impact of control measures. It is also important to take into account other economic sectors likely to be affected, including the general population and indirect concerns such as human health, societal and environmental impacts.

Both assessments were conducted by EAC teams comprising 5 experts from each EAC Partner State, except for South Sudan which had already been considered among the Intergovernmental Authority on Development (IGAD) Countries in a another study, 4 veterinarians from Veterinary Public Health, Veterinary Laboratory, Field Epidemiology and Wildlife Health, and one Public Health (zoonoses) expert from Ministries responsible for Public Health. These teams were supported by experts from the EAC Secretariat, AU-IBAR and Phylum who moderated discussions and provided clarifications on various aspects and interpretations of the tool.

All the information from these assessments was captured in a computerised model (Excel Sheet) that contains formulae and other linkages to help with computations. Data from the characterisation of the diseases was transferred to the iterative table where automated computation takes place to produce the weighted list of priority diseases. Both qualitative and quantitative elements of

prioritisation for the different diseases and specific criteria generated in three categories, namely absent diseases, present diseases with known epidemiology and diseases with unreliable local epidemiological knowledge as well as the corresponding control strategies that should be developed and implemented among other possibilities. The output from this tool was subjected to a comparative and interpretative process involving interdisciplinary experts and local decision makers, and integrating the different components of local geopolitical and socio-cultural orientations.

After going through the assessments, all the teams presented their findings in plenary for discussion during which they responded to questions on their country results from other professionals and moderators. Further, every team was tasked to organise discussions with other professionals and their seniors in their respective countries before arriving at a final result. Two to three weeks were provided for this process, after which all sent their final comparative tables to AU-IBAR for compilation. The regional perspective of priority diseases was derived from the top ranked diseases of the partner states, with a bias to aspects of disease control that require cross-border

interventions.

Results

Following a regional prioritisation exercise where all partner states and supporting institutions (EAC Secretariat, AU-IBAR and Phylum) took part, the most important diseases in the region were assessed and prioritised for intervention as presented in Tables 1 and 2. The priority diseases in order of importance are: African Swine Fever (ASF), Foot and Mouth Disease (FMD), Rift Valley Fever (RVF), Bovine Tuberculosis (BTB), Brucellosis, Contagious Caprine Pleuropneumonia (CCPP), Peste des Petits Ruminants (PPR), Sheep and Goat Pox (S&GP), Newcastle Disease (ND), Lumpy Skin Disease (LSD), Contagious Bovine Pleuropneumonia (CBPP), East Coast Fever (ECF), & Rabies. The important exotic diseases are: Highly Pathogenic Avian Influenza (HPAI) and exotic FMD serotypes.

Although Trypanosomiasis was not ranked among the priority diseases, it remains an important disease in the region especially in Uganda and Tanzania and some parts of Kenya. Tables 3, 4 and 5 outline the required regional national interventions.

Table 1: Prioritisation of major diseases in the EAC region (except South Sudan) and Implication for the EAC Region

Disease	Disease Score					EAC Score	EAC Rank
	Burundi	Kenya	Rwanda	Tanzania	Uganda		
ASF	9.5	5.9	10	10	10	9.80	1
FMD	8.0	5.0	10	9.0	7	7.80	2
RVF	10	6.0	7.0	8.0	4.5	7.10	3
BTB	5.8	3.6	6.0	9.0	9.9	6.86	4
Brucellosis	5.7	4.0	6.0	9.3	8.5	6.7	5
CCPP	5.0	4.0	9.0	7.6	7.7	6.66	6
PPR	6.3	3.8	6.0	9.4	7.1	6.52	7
S&GP	7.7	1.4	8.0	9.1	6.1	6.46	8
ND	6.0	3.0	8.0	8.1	6.4	6.38	9
LSD	8.4	1.8	9.0	5.8	6.8	6.30	10
CBPP	2.3	3.5	8.0	6.1	4.6	4.90	11
ECF	4.9	1.6	10	3.9	-	4.04	12
Rabies	5.3	3.0	6.0	4.7	-	3.8	13

ASF= African Swine Fever; FMD=Foot and Mouth Disease; RVF= Rift Valley Fever; BTB= Bovine Tuberculosis; CCPP= Contagious Caprine Pleuropneumonia; PPR = Peste des petits ruminants; S& GP=sheep & Goat Pox; ND= Newcastle Disease; LSD= Lumpy Skin Disease; CBPP= Contagious Bovine Pleuropneumonia; ECF= East Coast Fever

Table 2: Prioritisation of Exotic diseases

Disease	Disease Rank					EAC Rank
	Burundi	Kenya	Rwanda	Tanzania	Uganda	
HPAI	2	2	1	1	1	1
FMD EXS	1	1	2	2	2	2

HPAI= Highly Pathogenic Avian Influenza; FMD EXS= Foot and Mouth Disease Exotic Serotypes

Table 3: National Strategies

Diseases	Required Control Actions per country				
	Burundi	Kenya	Rwanda	Tanzania	Uganda
ASF	Enforce existing Sanitary Policy ; intensify epidemio-surveillance Sensitize pig farmers and strengthen advocacy.	No vaccine & treatment; Surveillance and disease outbreak investigations; Licensing pig producers; Enforce the existing law	Disease is absent; enforcement of Animal Health law; Strengthen border and internal controls	Disease not a priority, but control according to the national animal Health laws and regulations	Enforce the national animal health law; movement control and good animal husbandry practices; a nation strategy needed
FMD	Put in Place National FMD Control strategy and Contingency Plan; intensify Epidemio-surveillance and disease monitoring. Enforce the sanitary law. Comply with OIE-FAO-AU-FMD Pathway for Progressive FVD Eradication	Implement the draft FMD Control strategy. Target Mass vaccination, movement control, surveillance and Zonal cleaning of the country from the disease beginning from the Dairy Zone Comply with OIE- FMD Pathway for Progressive FVD Eradication	Strengthening control at borders, and internal control posts, diagnostic capacity, Enforcement of animal health law livestock movement, -stamping out Comply with OIE- FMD Pathway for Progressive FVD Eradication	Intensify epidemio-surveillance & strategic vaccination in frequently affected areas, for Progressive FVD Eradication	Implement the national Risk-Based Strategic Plan which aims at maintaining a zero circulation of FMD serotypes to enable the country apply for an infection-free status from the OIE
RVF	Develop early warning system; implement sustainable epidemio-suveillance strategy to reduce the risk of introduction; Contingency Plans to prepare for outbreaks	Surveillance, Public health education. Routine Mass vaccination in high and medium risk areas. Forecasting and climate models for the occurrence of disease;	Develop early warning system; vaccination (before rainy season) in risky zones; Intensify epidemio-surveillance;	Improve vaccine supply; early warning; enforce animal health laws to comply with OIE sanitary requirements	High priority disease; recent outbreaks call for Contingency Planning and preparedness, and enforcement of sanitary laws including vector control.

Diseases	Required Control Actions per country				
	Burundi	Kenya	Rwanda	Tanzania	Uganda
BTB	The national control program exist only in public health. Enforce the existing Sanitary policy exist for animals	a common control strategy for animal and public health Surveillance with modified stamping out.	Surveillance with modified stamping out.	No policy; need for epidemio-surveillance to determine the extent of the disease	Surveillance with modified stamping out.
Brucellosis	Public awareness; Undertake strategic mass vaccination campaign of young stock; Intensify epidemiological surveillance to map the disease.	Discussions on going on development of an integrated control strategy. Public health education Surveillance selective vaccination in high value dairy herds and selective culling	Lab testing + Culling positive cases Vaccination of replacement stock (young heifers)	Institute and enforce the national veterinary & public health sanitary regulations.	Need for a national policy and strategy mass vaccination of yearling calves
CCPP	Institute sustainable epidemio-suveillance to reduce the risk of introduction; movement control; and vaccination in high risk zones	Mass vaccination: movement control; perform risk based surveillance and zoning.; and operationalize the draft CCPP control strategy			Screening and mass vaccination in high risk areas
PPR	Undertake strategic mass vaccination campaign in high risk areas;Intensify epidemiological surveillance; Control of animal movements; Operationalize the animal sanitary policy &	Mass vaccination; movement control; risk based surveillance and zoning; and operationalize the national PPR control	Enforcement of animal health law; Strengthen controls at borders, and internal control posts; strengthen epidemio-surveillance & diagnostic capacity & vaccinate in	Improve vaccine supply; carry out strategic mass vaccinations; sensitise communities and strengthen epidemio-surveillance. Operationalise the national PPR strategy	Determine the epidemiological status of the disease in the country; zone according risks; mass vaccination for 3 yrs where disease prevalence is high; a National PPR Control

Diseases	Required Control Actions per country				
	Burundi	Kenya	Rwanda	Tanzania	Uganda
	the National PPR strategy		high risk areas. Operationalise the National PPR strategy.		Strategy is in place & should be operationalised
S&GP	Strengthen epidemio-suveillance intensified vaccination and movement control . s;	Strategic vaccination and Movement control	Strategic vaccination and Movement control	Strategic vaccination and Movement control	Strategic vaccination and Movement control Strategic vaccination
ND	Systematic and regular vaccination regime in both commercial farms and in local chickens; improve biosecurity at commercial farms	Vaccine manufactured in the country and is available locally. Need to involve all stakeholders in immunization of especially the free range; and need for sustained control strategy adopted	Biosecurity measures at farm level; Public awareness; strategic vaccination; and strengthen diagnostic capacity.	Systematic and regular vaccinations in local and commercial chickens; strengthen the enforcement of the animal health laws.	Strategic vaccination of free range poultry; routine vaccination of commercial poultry and institute strict Biosecurity measures in commercial poultry farms.
LSD	Strategic vaccination; strengthen enforcement of the existing sanitary laws particularly movement controls during outbreaks	Strategic vaccination Movement control No strategy	Strategic Vaccination; enforce movement control during outbreaks	Strategic vaccinations in affected areas; quarantine measures .	Strategic vaccination in affected areas; movement control and vector control.
CBPP	Sustainable suveillance; institute sanitary & prophylaxis measures such as quarantine and control of animal movements; mass vaccination in high risk zones	Risk based surveillance and zoning; Massive vaccination in infected zones; Movement control Selective culling in uninfected zones; and operationalize the draft contingency in place	Strategic Vaccination; Control of livestock movements Strengthen surveillance and diagnostic capacities Stamping out	Implement the national contingency plan; comply with OIE sanitary standards; improve vaccine supply for strategic vaccination	Mass vaccination in high risk areas; movement control; institute inspection at slaughter to determine the carriers

Diseases	Required Control Actions per country				
	Burundi	Kenya	Rwanda	Tanzania	Uganda
ECF	Sensitize communities ; Strengthen vectors control ; instate infection and treatment (IT) vaccine strategy especially in calves; and strengthen the quality control of the drugs and acaricides.	Institute strategic vaccination using the Infection-Treatment tool for ECF vaccine; Implement rational acaricide use and integrated pest-management	Strategic vaccination using the Infection-Treatment tool for ECF vaccine in the dairy herds Implement the tick control measures; rational acaricide use and integrated pest-management	Strategic vaccination using the Infection-Treatment tool for ECF vaccine; Implement rational acaricide use and integrated pest-management	Strategic vaccination using the Infection-Treatment ECF tool Implement the tick control policy; rational acaricide use and integrated pest-management
Rabies	Engage more stakeholders in order to improve the vaccine supply and vaccination coverage; Strategic mass vaccination; and sensitize local administration and communities	Compulsory annual mass canine vaccinations; provision of post exposure prophylaxis in and public humans; improved surveillance for rabies in dogs and humans	Annual Canine vaccinations; Destruction of stray dogs; creation of public awareness; and improve surveillance and diagnostic capacity.	Intensify surveillance in canines and humans; Mass canine vaccinations; strengthen diagnostic capacity and implement the national rabies strategy	Mass vaccination of dogs for 3 years; promotion of exposure prophylaxis for children and vulnerable groups.
HPAI	Strengthen laboratory capacity and epidemio-surveillance in wild birds and reporting; Need for HPAI Control Strategy, Contingency & Preparedness Plan	Surveillance in wild birds and in live bird markets and reporting Need for HPAI Control Strategy, Contingency & Preparedness Plan	Strengthen laboratory capacity and epidemio-surveillance in wild birds and reporting. Need for HPAI Control Strategy, Contingency & Preparedness Plan	Build capacity for HPAI surveillance and laboratory diagnosis. Need for HPAI a Control Strategy, Contingency Preparedness Plan	Improve laboratory capacity for the diagnosis of HPAI and strengthen epidemio-surveillance in wild birds; improve reporting. Need for HPAI Control Strategy, Contingency & Preparedness Plan

Diseases	Required Control Actions per country				
	Burundi	Kenya	Rwanda	Tanzania	Uganda
FMD EXS	Strengthen laboratory capacity and epidemio-surveillance and reporting	Surveillance and stamping out in index herd	Surveillance and stamping out in affected herds; quarantine and strict movement control	Strengthen Epidemiological surveillance; institute a comprehensive Control Strategic plan; strengthen disease reporting system from the grass & prompt response mechanism; Put in place a Contingency and preparedness Plan.	Develop contingency plans; Institute border Controls; procure Vaccine Framework contracts; and formulate and enforce a National slaughter and Compensation Policy.

ASF= African Swine Fever; FMD=Foot and Mouth Disease; RVF= Rift Valley Fever; BTB= Bovine Tuberculosis; CCPP= Contagious Caprine Pleuropneumonia; PPR = Peste des petits ruminants; S&GP=; ND= Newcastle Disease; LSD= Lumpy Skin Disease; CBPP= Contagious Bovine Pleuropneumonia; ECF= East Coast Fever; HPAI= Highly Pathogenic Avian Influenza; FMD EXS= Foot and Mouth Diseases Exotic Serotypes

Table 4: Implication for the EAC Region

Disease	Regional Strategies
ASF	High priority disease & of high risk. Needs a strong regional strategy to protect the clean territory and to minimize impact in the affected Partner States
FMD	A high priority disease that regionally affects trade. Could benefit from a well coordinated regional control strategy
RVF	A high priority zoonotic disease that regionally affects trade. Could benefit from a coordinated regional control strategy based on One Health system
BTB	Priority endemic zoonotic disease whose epidemiological status should be adequately assessed in the region.
Brucellosis	Endemic priority zoonotic disease. Requires concerted national and regional efforts
CCPP	Fairly important trade disease for the region with some clean Partner States that require regional measures to protect their free Partner States
PPR	A high priority disease regionally that could benefit from a coordinated regional control strategy based on the Continental and Global Strategies
S&GP	Important disease .A regional strategy to roll back the disease due to its impact on food security &trade
ND	A high priority disease in the region whose control strategy can also serve as a proxy for the prevention of HPAI in the region
LSD	Priority disease which affects beef production and the leather industry
CBPP	Important disease but not such a high priority. However, vigilance should be maintained in the partner States where the disease has been controlled

Disease	Regional Strategies
ECF	Has low regional significance; Scores high as a constrain to production at farm level; Should be taken seriously due to the current increase of tick acaricide resistance in the region
Rabies	Important endemic zoonotic disease of high priority. Important to share experience and adopt a regionally coordinated strategy under on One Health arrangements

ASF= African Swine Fever; FMD=Foot and Mouth Disease; RVF= Rift Valley Fever; BTB= Bovine Tuberculosis; CCPP= Contagious Caprine Pleuropneumonia; PPR = Peste des petits ruminants; S&GP= sheep & Goat pox; ND= Newcastle Disease; LSD= Lumpy Skin Disease; CBPP= Contagious Bovine Pleuropneumonia; ECF= East Coast Fever

Table 5: Priority Exotic Diseases Implication for the EAC region

Disease	Strategic actions for the EAC Region
HPAI	At national level, coordinate and strengthen border controls and development of contingency plans for early detection and emergency response; enhance capacity building to ensure adequate enforcement. A regional strategy and contingency& Preparedness Plan for the prevention of HPAI could use New Castle Disease as a proxy for the establishment of prevention and control mechanisms.
FMD ExS	Implement the national FMD Control strategies in line with the Global Progressive FMD control and eradication. Need to coordinate national level efforts and strengthen border controls and development of contingency plans for early detection and emergency response; enhance capacity building to ensure adequate enforcement. Enforce a regional strategy and contingency& Preparedness Plan for the Progressive control of FMD including exotic serotypes

HPAI= Highly Pathogenic Avian Influenza; FMD EXS= Foot and Mouth Diseases Exotic Serotypes

Discussion

The EAC regional exercise for the prioritisation of animal diseases including those transmissible to humans was the first of its kind in Africa. Although Africa has among the highest burdens of disease in both animal and human populations globally, disease control efforts have largely remained weak and disjointed. This has been caused by a myriad of reasons including but not limited to weak veterinary and human health systems, under-resourcing of disease prevention and control services, limited research and technology adoption, under-development of the main animal producing areas occasioning poor access to essential services such as veterinary and human health care and lack of tools for evidence based decision making in resource allocation and policy formulation.

Outcomes from the regional exercise indicated that all the EAC Partner States have specific priority diseases, many of which are also present in other countries, but do not

necessarily have the same importance. This is expected considering the differences in production systems, economic value of different species of animals and the local importance of livestock. Whether animals are reared for domestic consumption or trade and the types of animals under consideration are important causes of variability to the importance of different diseases. For instance, although ASF scored highly across Burundi, Kenya, Tanzania and Uganda, only Burundi and Uganda classified it among the top five diseases due to the importance of the pig sub-sector on local food security. Uganda has the largest pig population in the region (1) kept in a free range system of management. The country's population relies heavily on pork for food security and livelihoods and most of the production is consumed locally. Similarly, Burundi utilizes its significant pig population for local consumption and livelihoods. Due to pressure on land, pig production plays a highly significant role as its production does not require large land sizes. South Sudan was not included in this study as

it was assessed in another study for the IGAD Member States.

While most interventions will take place at Partner States level, the analyses performed by the Partner States and the EAC Secretariat representatives showed that the EAC could have strong value addition in the implementation of regional animal health control programmes targeting diseases with a regional presence/significance (9,10,11) These include the following;

1. Exotic diseases such as HPAI and exotic serotypes of FMD by coordinating the strengthening of border controls and development of contingency plans for early detection and emergency response, and capacity building to ensure adequate enforcement. A regional strategy for the prevention of HPAI could use New Castle Disease as a proxy for the establishment of prevention and control mechanisms;
2. The regional coordination of national animal health programmes on diseases with regional significance such as: FMD (circulating serotypes), RVF, PPR, ND and ASF. Usually such TADs cannot be effectively managed by one country alone due to their epidemiological dynamics and impacts on trade;
3. Control of regionally important neglected zoonoses such as brucellosis, bovine tuberculosis and rabies that impact on livestock trade and public health and are endemic in the region;
4. Regional support provided through:
 - a. Common regional strategies, programmes, regulations and procedures to control diseases,
 - b. Development of coordinated policies for border control: strengthening of border posts, optimisation of the network of border posts, common procedures etc.,
 - c. Consultation at regional level with stakeholders, especially concerning safe regional trade,
 - d. Complementary resources for vaccination campaigns and compensation programmes,

- e. Coordinated regional initiatives such as vaccines banks, shared infrastructure and services e.g. diagnostic laboratories and veterinary expertise,
- f. Establishment of regional (virtual) emergency response teams, capabilities, logistical support structures and material stock piles.
- g. Joint simulation exercises for TADs and Zoonoses
- h. Joint programmes to address TADS and Zoonoses control in Transboundary ecosystems like the Kagera ecosystem, Karamoja –Turkana ecosystem and the Masai Mara-Serengeti Ecosystem

Conclusion

This list of priority diseases created a clear opportunity for the EAC region to adopt measures for the systematic control and progressive eradication of animal diseases on the basis of evidence of their importance at both country and regional levels. Stakeholders and decision makers now have a good tool for determining the actions to take, the roles for different actors, the resources required and the milestones they need to achieve common objectives. Diseases that have a strong impact at national rather than regional levels obviously warrant investment at the country level, with contiguous countries effective pre-emptive measures to prevent incursion or spread. Diseases with a strong regional impact such as FMD, PPR, SG&P, ND, Rabies and Brucellosis among others require regional level programs and strategies with the participation of all Partner States and other actors to roll back. The list of diseases has significance in a number of areas including;

1. Evidence based decision making regarding required actions;
2. Roles of different stakeholders based on the nature of disease and impacts – some diseases only warrant private level investment while there are those that need public common good approaches;
3. Some diseases have ongoing control programs but may require review to

- improve while others have not and may need to be initiated; and
4. The nature of some diseases require more emphatic approaches such as stamping out/ sustained mass vaccination with stamping out/ zoning/compartimentalization
 5. Some diseases (FMD, PPR, ASF, HPAI) are already the subject of global control programs (12) and the region and the Partner States need to embrace global strategies to deal with them –

As the control of transboundary animal diseases and zoonoses is a public good, the critical needs of veterinary and public health systems must be addressed in efforts to achieve sustainable control. Thus, the list of priority diseases could also be used to program investment in strengthening the capacities of the VS by addressing critical competencies that are key success factors for their effective control. For this, the EAC secretariat has an important role to play in tandem with its other programs to promote integration and safe regional trade. It is deemed necessary therefore that the secretariat endorses the priority list of diseases and fully mainstreams their management into its institutional programs.

This list includes some diseases recognized for their international importance (FMD, PPR, HPAI). International programmes developed by development and technical institutions such the OIE and FAO should aim to support the regional policy developed by EAC and its Member countries. Control programmes for that will need to be developed to support the strengthening of some critical competencies of veterinary services may include:

1. *Vaccination campaigns* - should be used to develop the veterinary network and the passive surveillance for a full coverage of the territory;
2. *Control of exotic diseases* - should give the opportunity to modernise the border inspection and the control of movements;
3. *The development and implementation of the different contingency plans* - should strengthen the capacity for early detection

- and emergency response (12, 13) ; and
4. *Control of Zoonoses* - should provide opportunity to strengthen inter-sectoral collaboration and the one health approach (14).

Conflict of interest

There was no conflict of interest identified

Ethical standards

The study involved review of secondary data and reports generated during routine animal and human disease surveillance in the EAC Partner States. There was no direct contact with human and animal subjects. This was part of the routine surveillance activities which are exempted from Institution Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought in all the EAC Partner States. The study has many benefits to the human and animal populations by guiding policy and resource allocation for prevention and control of the diseases in the EAC Region.

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THE EFFECT OF WIND SHEAR STRESS IN WATER QUALITY IN NET CAGE FISH FARMING IN A LAKE OPEN WATER COURSE – THE CASE STUDY OF NHAMBAVALE LAGOON, GAZA PROVINCE, MOZAMBIQUE

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Abstract

Fish farming in net-cages in open water courses entails high stocking density which is associated with a high load of pollution. The water renewal in these systems is through either advection or turbulent diffusion or both. This study examined the role of wind shear stress in stimulating vertical turbulent diffusion, water renewal and improving water quality in net-cage culture of tilapia species *Oreochromis mossambicus* in open water courses in Nhabavale Lagoon, using Dissolved Oxygen (DO) and the Biochemical Oxygen Demand (BOD) as water quality indicators. Water renewal was assumed to be due to turbulent diffusion driven by wind shear stress. DO and BOD were measured inside the cages and at a remote place outside the cages at 08:00 hours, over a period of one month (September - October 2014). Statistical analyses of variance, ANOVA, with confidence intervals were performed to compare the parameters inside and outside the cages. The water quality parameters were linearly regressed with wind shear stress to investigate the influence of this in the renewal of water in the net-cages. The results indicated that the water temperatures were at the lower limit of the optimum range for tilapia growth, that there were significant differences between the water quality parameters OD and BOD but no significant differences with respect to the water temperature inside and outside the cages; the OD and BOD were positively and negative linearly correlated with the wind shear stress, respectively, with the coefficient of goodness of fit $r^2=0.20$ for both, and coefficients $a=0.1$ ($p=0.21$) and $a=-0.06$ ($p=0.02$) for OD and BOD, respectively. It was concluded that wind shear stress stimulated directly the dissolution of oxygen in water, promoted turbulence and vertical mixing, contributing by 20% to water renewal and reduction in the density of organic matter, which ultimately resulted in improved water quality in the net-cages.

Key words: Net-cages, Dissolved Oxygen, Biochemical Oxygen Demand, Wind force, Turbulent diffusion, Water quality

EFFET DE LA CONTRAINTE DE CISAILLEMENT DU VENT SUR LA QUALITÉ DE L'EAU DANS LA PISCICULTURE EN CAGES EN MILIEU LACUSTRE OUVERT - L'ÉTUDE DE CAS DE LA LAGUNE DE NHAMBAVALE DE LA PROVINCE DE GAZA AU MOZAMBIQUE

Résumé

La pisciculture en cages en milieu lacustre ouvert implique une forte densité de poissons qui est associée à une charge de pollution élevée. Le renouvellement de l'eau dans ces systèmes se fait soit par advection, soit par diffusion turbulente, soit les deux. Cette étude a examiné le rôle du stress dû au cisaillement du vent dans la stimulation de la diffusion turbulente verticale, le renouvellement de l'eau et l'amélioration de la qualité de l'eau dans l'élevage en cage d'espèces de tilapias *Oreochromis mossambicus* dans des cours d'eau ouverts dans la lagune de Nhabavale, en utilisant l'oxygène dissous (OD) et la demande biochimique en oxygène (DBO) comme indicateurs de la qualité de l'eau. On a pris comme hypothèse que le renouvellement de l'eau

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était dû à la diffusion turbulente induite par le cisaillement du vent. L'OD et la DBO ont été mesurées à l'intérieur des cages et dans un lieu reculé à l'extérieur des cages à 08h00, sur une période d'un mois (septembre à octobre 2014). Des analyses statistiques de variance, ANOVA, avec des intervalles de confiance ont été effectuées pour comparer les paramètres à l'intérieur et à l'extérieur des cages. Les paramètres de qualité de l'eau ont été régressés linéairement avec la contrainte de cisaillement du vent afin d'en étudier l'influence sur le renouvellement de l'eau dans les cages en filet. Les résultats ont indiqué que les températures de l'eau se situaient à la limite inférieure de la plage optimale de croissance du tilapia, qu'il existait des différences significatives entre les paramètres OD et DBO de la qualité de l'eau, mais pas de différence significative en ce qui concerne la température de l'eau à l'intérieur et à l'extérieur des cages ; la DO et la DBO étaient corrélées positivement et négativement linéairement avec la contrainte de cisaillement du vent, respectivement avec le coefficient de qualité de l'ajustement $r^2 = 0,20$ pour les deux, et les coefficients $a = 0,1$ ($p = 0,21$) et $a = -0,06$ ($p = 0,02$) respectivement pour l'OD et la DBO. Il a été conclu que la contrainte de cisaillement du vent stimulait directement la dissolution de l'oxygène dans l'eau, favorisait la turbulence et le mélange vertical, contribuant de 20% au renouvellement de l'eau et à la réduction de la densité de matière organique, ce qui aboutissait à une amélioration de la qualité de l'eau dans les cages en filet.

Mots clés : cages en filet, oxygène dissous, demande biochimique en oxygène, force du vent, diffusion turbulente, qualité de l'eau

Introduction

Fish breeding in net cages is an intensive venture involving high stocking density. It could be highly profitable. However, the high amount of waste generated in the process can cause rapid deterioration of water quality in the cages and in the surrounding waters. Therefore, in such systems, constant renewal of water is required (Guo *et al.*, 2009; Gao *et al.*, 2005; Guo and Li, 2003). If the cages are placed in open water courses the renewal of water can be granted by the advection of water from the surroundings, if there is a stream such as in estuaries and tidal inlets or by turbulent diffusion in enclosed systems such as lakes (Hamblin and Gale, 2002).

Though the turbulent diffusion is a slow process compared with advection, it provides both the exchange of water through the mixing process and the absorption of atmospheric oxygen through surface diffusion, a process that is stimulated by wind shear stress and ripple on the surface (Kutty, 1987); given that the molecular diffusion of gases in water is very minor, most of the dissolved oxygen in natural waters is transferred from the atmosphere through agitation of water

as noted by Michaud (1991), with wind shear stress as the natural agitation mechanism. Several studies have shown that more oxygen dissolves in water when there is wind stirring; as the waves create more surface area, more diffusion can occur, thus, gas exchange velocity between the atmosphere and open water bodies increases with wind speed (Banks, 1975; Crusius and Wanninkhof, 2003; Liss and Merlivat, 1986). However, there are few published studies attempting to estimate the contribution of wind force in water renewal in aquaculture systems.

This study examined the contribution of wind shear stress in promoting water exchange, and hence, improving water quality in net-cages of tilapia fish culture of species *Oreochromis mossambicus* in open water course in a lake. Nhabavale Lagoon, in southern Mozambique was used as a case study.

Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD) are used as indicators of the water quality. The DO is a necessary element to aquatic life; it is required for animal respiration, photosynthesis and in the biodegradation of organic matter. It is therefore, also used as an indicator of the water quality (EPA, 2012; Murphy, 2007; Carter, 2005). The

BOD is one of the most important parameters of water quality as it is indicative of the amount of oxygen, dissolved in the water, required for microorganisms to decompose organic matter, often used as the organic matter concentration index in water. It is thus a measure of the degree of organic pollution of a water (EPA, 2012; EPA, 2013; Bassoi and Guazelli, 2004).

In this study it was assumed that the water quality in the net cages was affected by the metabolic processes and by the organic matter resulting from fish excrement and remains of fish feed, and that the renewal of the water was continuous and driven by the turbulent diffusion due to wind shear stress. Knowledge about the natural water exchange mechanisms, in open water course aquaculture systems, is needed for determination of the carrying capacity of the system and assuring adequate water quality for healthy fish growth, performance and avoiding diseases.

Material and methods

Place of study

The research was carried out at a tilapia production facility in Nhambavale Lagoon, in Chidenguele Administrative Post, Mandlakaze District, Gaza Province, Mozambique, located at Latitude 24° 54' 28, 82" S and Longitude

34° 17' 34.88" E (Figure 1). The Nhambavale Lagoon is about 35 km long and 1.5 km wide. The depth is not known but at the location of the experimental site, it ranged from 0 m to 2.5 m. According to the climate classification of Wladimir Köppen, the climate of Mozambique is classified as equatorial savannah with dry winter, with two distinct seasons: summer or wet season and winter or dry season (Kottek et al., 2006). The climate of southern Mozambique is influenced by the warm Mozambique current and the Indian Ocean Sub-tropical Anticyclone System of the Southeast Trade Wind Zone (Sætre and Jorge da Silva, 1984). The average monthly and annual temperatures are between



Figure 1: Location of the study site.

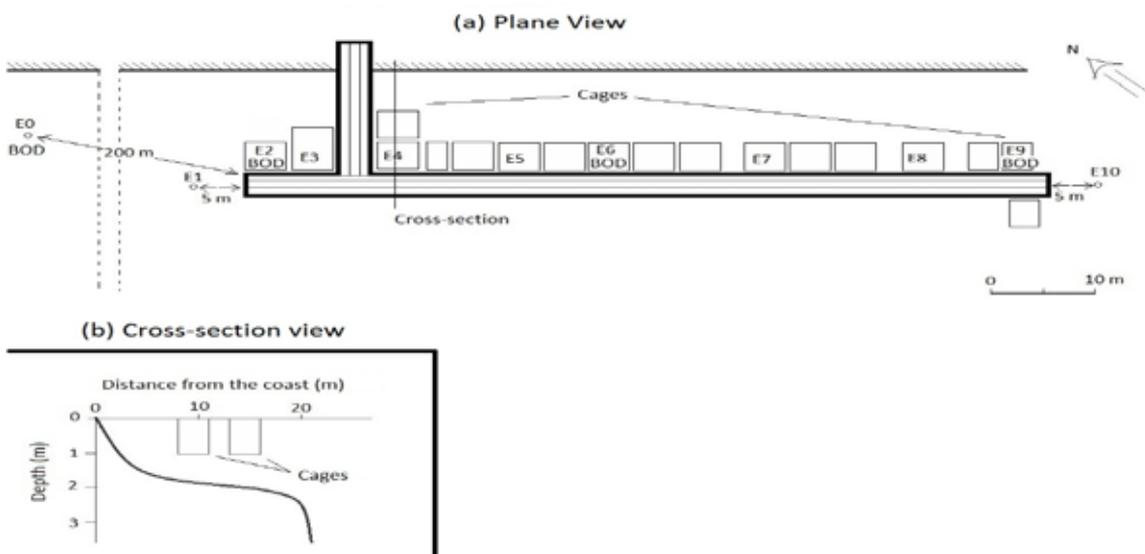


Figure 2: Delineation of the experiment and location of the stations where data were collected: (a) Plane view and (b) cross-section view.

Table 1: Type and size of the cages and storage and initial biomass density in the cages where the measurements were made.

Station ID Category	E2	E3	E4	E5	E6	E7	E8	E9
Mesh size (mm)	19	1	19	19	19	1	1	19
Volume of the cages (m ³)	8	16	16	16	16	16	16	8
No of the fish stocked	1500	1500	1500	1500	1500	3000	1500	1500
Individual initial fish weight (g)	80	80	118	85	111	111	113	93
Total initial biomass (kg)	120	120	177	127	166	330	169	139
Initial stocking density (N m ⁻³)	187	94	94	94	94	187	94	187
Initial stocking density so (kg m ⁻³)	15.0	7.5	11.0	7.9	10.3	20.6	10.5	17.4

20°C and 28°C and above 24°C, respectively. Precipitation is unevenly distributed between November and March, with annual rainfall varying between 400 mm and 950 mm. The evapo-transpiration is generally greater than 1,500 mm. High temperatures and evapo-transpiration combined with poor levels of precipitation cause a deficit in water availability. The vegetation is predominantly herbaceous with shrubs and small trees. The geology is dominated by coastal sandstone, parabolic dunes and coastal lagoons.

Delineation of the experimental location and data collection

The production unit consisted of 18 net cages of 1.9 cm mesh size and few of 1 mm mesh size. The cages had dimensions varying between 4 m length, 2 m width and 1 m height; 4 m length, 3 m width and 1 m height and 10 out of the 18 cages had 4 m length, 4 m width and 1 m height. The cages were assembled together along the edge of the lagoon (Figures 2a), in a location whose depth was about 2.5 m (Figure 2b). The stocking density was on average 250 fish of about 100 g individual weight per cubic meter, making a total initial biomass of about 6,000 kg (Table 1). The fish were and fed using commercial fish feed AQUA – Plus, twice daily.

Determination of parameters

Water temperature and DO were measured in the net cages and in a remote place outside the culture site, at 0.5 m depth from the surface at mid-point in the cages, at 08:00 hours every day, during the period

from 22 September to 21 October 2014, at the following locations: E0, a distant site about 200 m from the net cages' installations; for comparison, E1, located about 5 m upstream of the net cages, and E2 to E9 inside the net cages. The water temperature and dissolved oxygen were measured by means of an electronic probe YSI - 550A, with a precision of 0.1 oC and 0.1 mg L⁻¹ for temperature and DO, respectively. Wind speed was measured continuously and averaged over 10 min prior to the determination of temperature and DO by means of a hand anemometer with precision of 0.01 m s⁻¹. The choice of measuring at 08:00 hours aimed at depicting the lowest oxygen value, expected shortly after the sunrise and before the photosynthesis activity, and to avoid the complications in the data interpretation that might arise with the production of oxygen in the water column due to light intensity during the day-time. Samples for BOD determination were also collected at 08:00 hours and for 12 days. BOD was determined by the dilution method that consists of measuring DO concentrations before and after 5 days incubation, and appropriately adjusted by the sample corresponding dilution factor, as described by Jouanneau *et al.*, (2014) and by Muller *et al.*, (2014).

Wind blowing over the sea surface exerts a stress on the ocean that imparts momentum. A similar process occurs in open water courses such as lakes and lagoons. The magnitude of this shear force per unit contact area is estimated through wind-shear or wind-

drag formulas that parameterize the shear stress as a function of the wind speed, obeying the quadratic drag law, at a certain height above the surface in the form:

$$\tau = C_D \rho_{air} \omega_h^2 \quad [1]$$

Where $\omega_h=2$ is the wind speed measured at 2 m above the lagoon surface in the present study, $\rho_{air} = 1.22 \text{ kg m}^{-3}$ is the density of air, $C_D = 0.0013$ is the dimensionless drag coefficient. Thus, wind shear stress was given in units of N m^{-2} , or Pascals (Pa).

Statistical analysis

Unifactorial analysis of variance (ANOVA), at 5% probability level, was applied to compare the quality of water inside and outside the net cages. The Tukey test was applied at 5% level for comparison of means. Linear regression of the water quality parameters with the wind shear stress was performed to establish the effect of the wind in the quality of water inside the net cages, using the MINITAB

statistical package.

Results and Discussion

Results

Time series of wind stress, water temperature, DO and BOD

Figure 3 shows the time series of wind shear stress, water temperature, DO and BOD measured at 08:00 hours. The wind shear stress varied from 0 up to 1 N m^{-2} . About 50% of the time it was 0; 25% of the time it was 0.1 N m^{-2} , and 0.2 N m^{-2} and 0.3 N m^{-2} were observed during 15% and 10% of the time, respectively and the remaining values, up to 1 N m^{-2} were recorded in less than 5% of the time. The temperature ranged between 17°C and 19°C , most of the time. This was at the lower limit of the optimum range for tilapia growth (Trewavas, 1982). On September 26th and October 8th 2014, the temperatures were higher and attained about 22°C . From October 13th to the end of the observation period, there was a slight tendency of temperature reduction, with the lowest temperature recorded of about

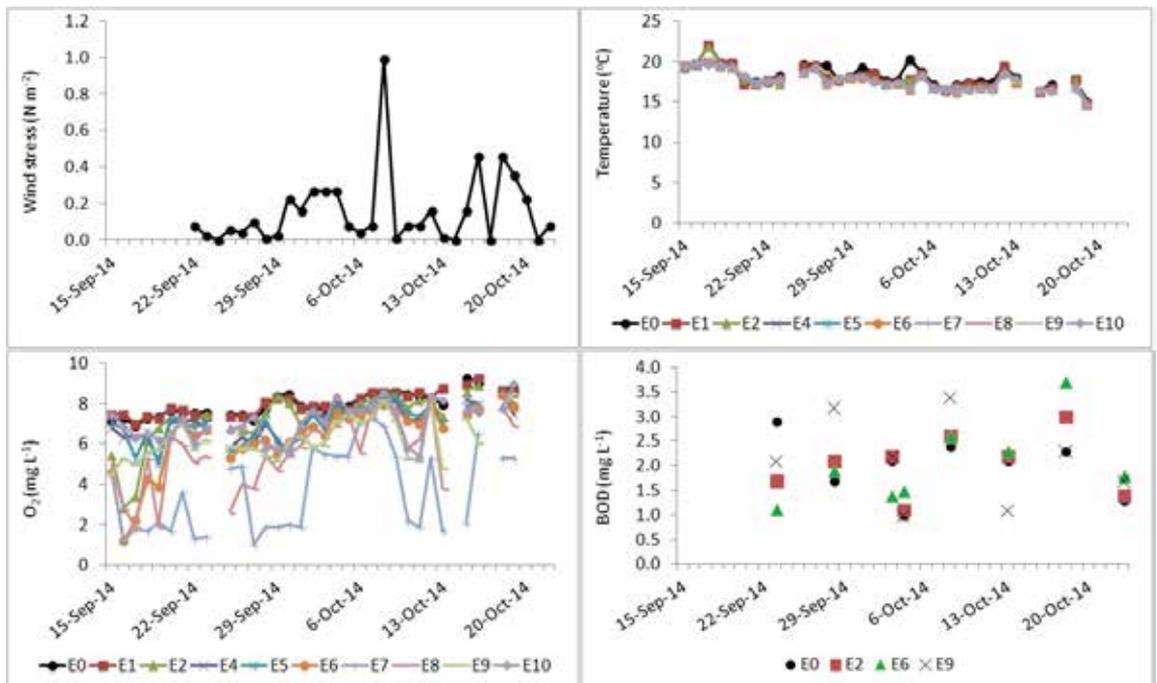


Figure 3: Time series of wind shear stress, water temperature, DO and BOD, measured at 8:00 o'clock at the tilapia aquaculture in net cages in Nhabavale Lagoon, Chidenguele, during 21 September to 21 October 2014.

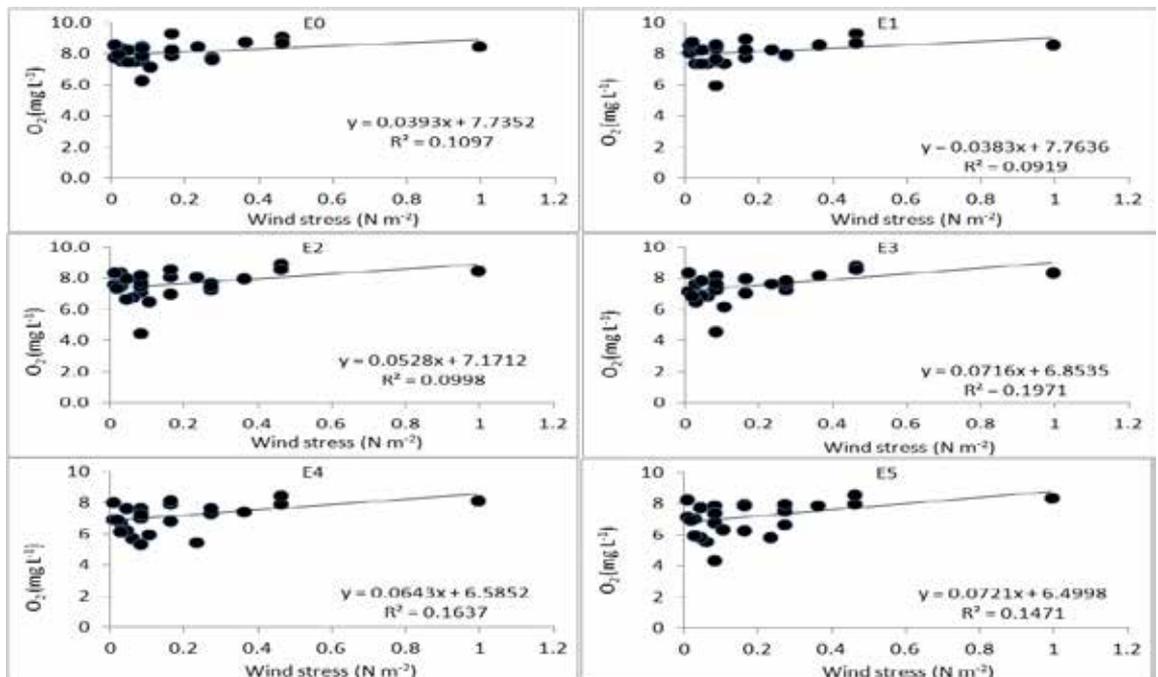
Table 2. Values of the similarity coefficient between the parameters measured at the stations inside the cages and those measured at Station E0, distant from the net, at the confidence interval .

Cage/station	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Temperature	0.006	0.005	0.000	0.013	0.013	0.009	0.010	0.004	0.022	0.014
DO	0.002	0.099	0.130	0.037	0.332	0.070	0.949	0.521	0.498	0.990
DBO		0.013				0.404			0.507	

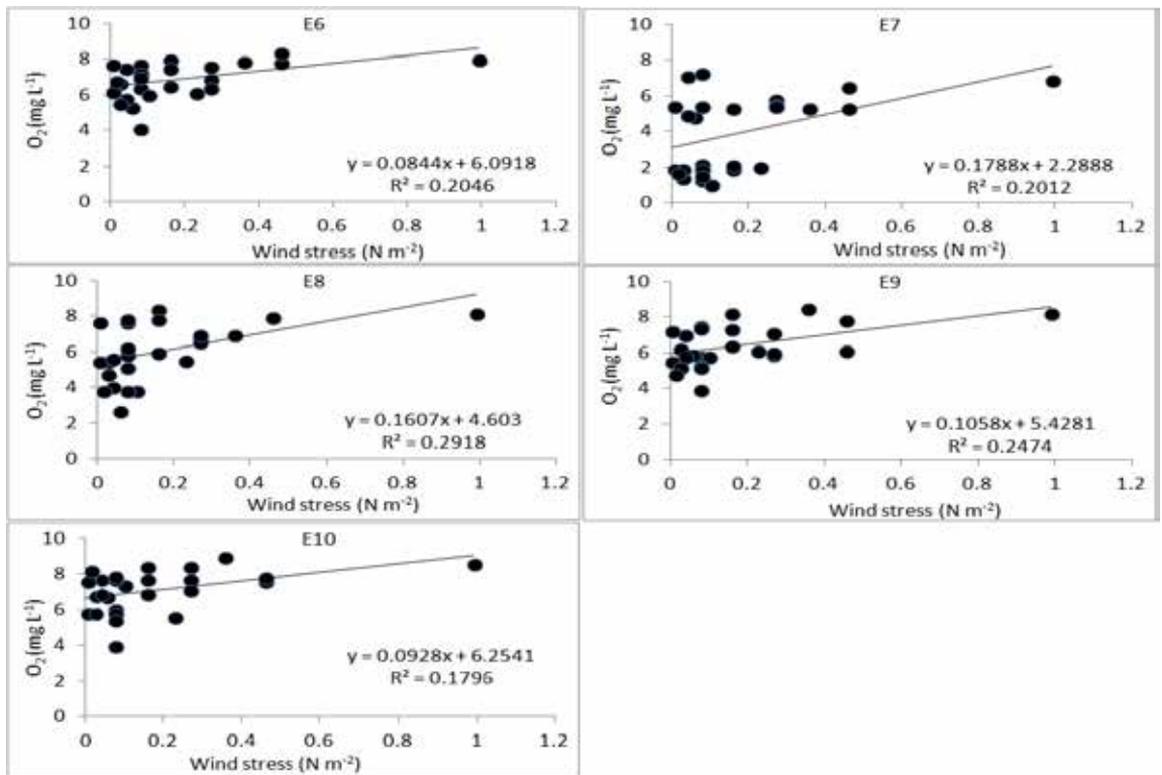
15°C. Dissolved oxygen varied between 6 mg L-1 and 9 mg L-1 in almost all the stations except at station E7 where values of less than 6 mg L-1 were recorded most of the time, and many values were below 2 mg L-1. At E1, E2, E6, E8 and E9 values of less than 6 mg L-1 were observed at the beginning of the experiment. Considering that these values were the lowest observed during the experiment, since the observations were made at 08:00 hours, it can be considered that the oxygen values were within the optimum range for tilapia growth throughout the experiment (Trewavas, 1982). It should be noted, however, that the net cages at stations E6 and E7 were constructed using mosquito nets with a mesh size of 1mm, which posed some restriction to water exchange in

the cages. The BOD values ranged between 1 mg L-1 and 3 mg L-1 in most of the stations, including at station E0 located far from the net cages. Higher values of BOD (greater than 3 mg L-1) were recorded at the stations inside the net cages E2, E6 and E9 which means there were higher concentrations of organic matter, representing a high content of organic pollution.

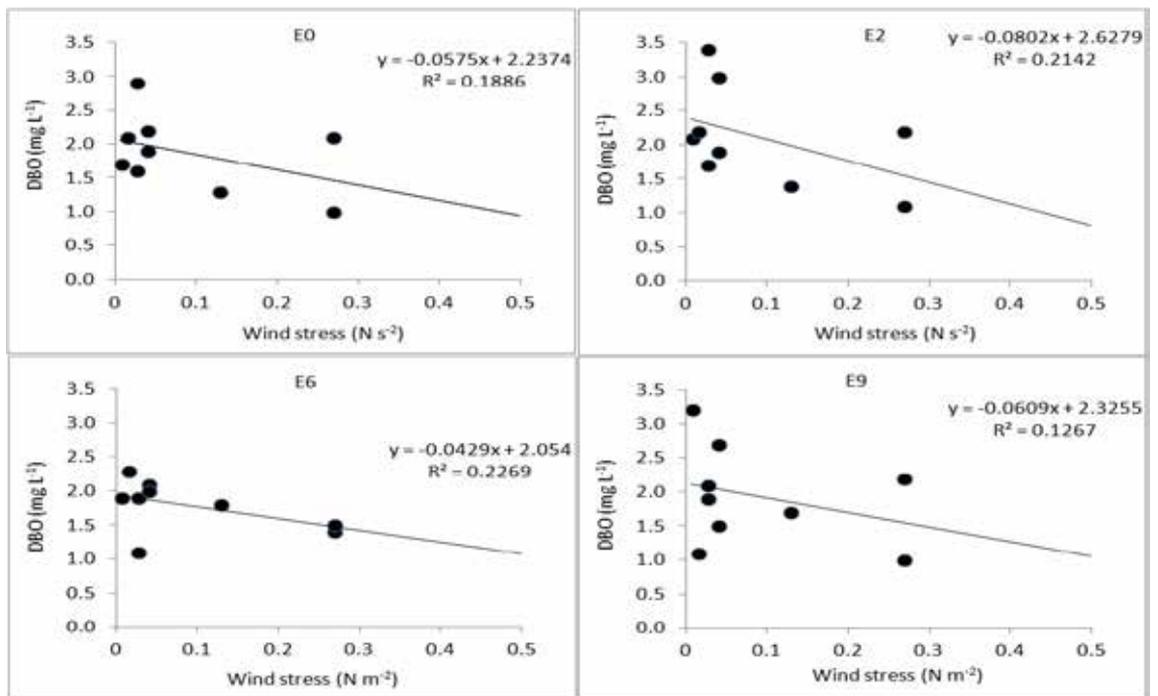
Table 2 presents the result of the statistical comparison between the parameters measured inside the net cages and those measured at the remote place away from the culture site (E0), at the confidence interval . There were significant differences between the DO and BOD measured in the cages and those measured at the site away from the cultivation



Figures 4a: Relationship between DO and wind shear stress measured at 8:00 o'clock at the tilapia aquaculture in net cages in Nhabavale Lagoon, Chidenguele, during 21 September to 21 October 2014.



Figures 4b: Relationship between DO and wind shear stress measured at 8:00 o'clock at the tilapia aquaculture in net cages in Nhabavale Lagoon, Chidenguele, during 21 September to 21 October 2014.



Figures 5: Relationship between BOD and wind shear stress measured at 8:00 o'clock at the tilapia aquaculture in net cages in Nhabavale Lagoon, Chidenguele, during 21 September to 21 October 2014.

site (E0). However, there were no significant differences between the water temperatures inside the cages and those measured at the remote place (E0).

The effect of wind shear stress on DO and BOD

Figure 4 shows the graphs of the relationship between DO content and wind shear stress measured at 8:00 in the net cages and in a remote station (E0). In all the graphs the DO content increases slightly with the wind shear stress. At the remote station (E0), away from the net cages the values were more diffuse, though the tendency of increase of DO content with the intensity of the wind shear stress is notorious. The coefficient of goodness of fit of the linear regression varied from $r^2=0.1$ to $r^2=0.29$, with the average values of $r^2=0.20$ and coefficient $a=0.1$ and $p=0.02$. Low values of about $r^2=0.1$ were observed in the remote station (E0) and in the two first upstream stations (E1 and E2), which leads to the understanding that in these stations there were no significant differences between the surface and subsurface DO content, hence, the vertical turbulent mixing brought by the wind shear stress gave no major changes in the DO content at the measured point.

Figure 5 shows the graphs of the relationship between BOD and wind shear stress measured at 08:00 hours in the cages and in a remote station (E0). The graphs show clearly that the BOD content reduced with the wind shear stress in all the stations, including the Station E0, away from the net cages. The coefficients of goodness of fit in the linear regression varied from $r^2=0.13$ to $r^2=0.23$, with average values of $r^2=0.20$ and coefficient $a = -0.06$ and $p = 0.2$.

Discussion

Variations in water temperature, DO and BOD

The relatively low water temperature values observed during this study (15-22°C) can be justified by the fact that measurements were taken in the morning, at 08:00 hours just after the Sunrise before the heating of the daytime, and also because the observation period

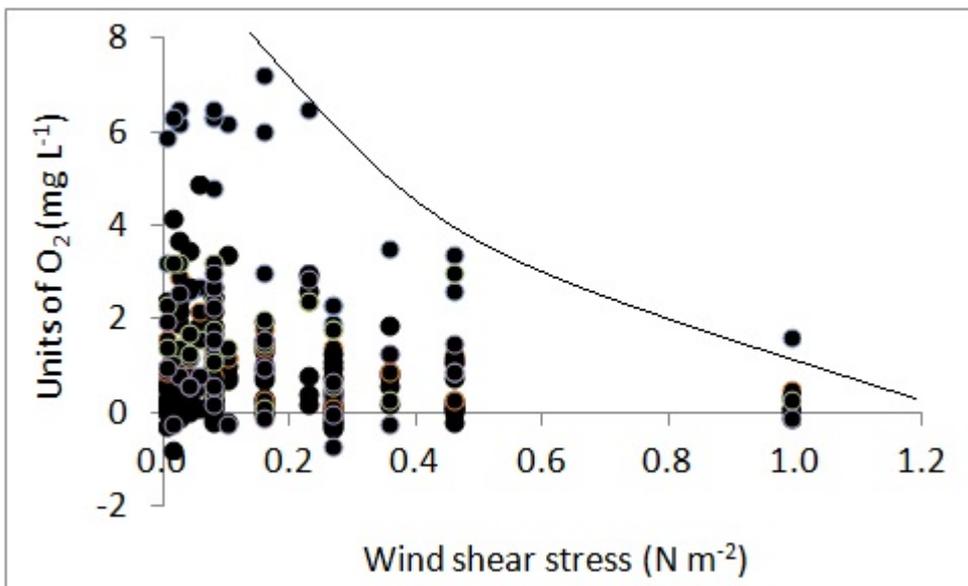
coincided with the transition between the winter and the summer (September-October). The values of DO content were between 6 mg L⁻¹ and 8 mg L⁻¹, occasionally reaching 9 mg L⁻¹ and 5 mg L⁻¹, in almost all the stations excluding stations E6 and E7, where in some cases the values of DO dropped to about 1 mg L⁻¹. These results are in agreement with those obtained by Mallasen *et al.*, (2012), who studied the water quality in the net cages of tilapia culture, in the Ilha Solteira reservoir in São Paulo, Brazil and obtained values between 5 mg L⁻¹ and 8 mg L⁻¹. The low values recorded in stations E6 and E7 may be associated with the high fish density and the fact that the net cages in these stations were constructed using mosquito nets with a small mesh size, of about 1 mm (Table 1), which restricted the renewal of water. The values observed in the other net cages were within the optimal range for tilapia growth. According to Trewavas (1982) the DO content for tilapia growth should not be less than 2 mg L⁻¹, and the ideal should be equal to or greater than 4 mg L⁻¹. The BOD values obtained in this study ranged from 1-2 mg L⁻¹ in the Station E0, far from the net cages and from 1-3 mg L⁻¹ in the stations E2 and E9 and 0.5-2 mg L⁻¹ in the Station E6. Thus, the oxygen demand in the oxidation of organic matter was much lower than the dissolved oxygen, even after the reduction of oxygen by the breathing processes at night. The BOD values obtained in this study were low indicating low levels of pollution, compared to those obtained by Mallasen *et al.*, (2012) who recorded values between 2 and 4 mg L⁻¹.

Relationship between wind shear stress and DO and BOD

The positive correlation between the wind shear stress and the DO in this study is in agreement with the finding by Banks (1975), and can be justified by the fact that the wind induces turbulence on the surface, which in turn increases the dissolution of oxygen in the superficial layers, which is in line with the findings by Crusius and Wanninkhof (2003) and by Liss and Merlivat (1986). DO, at the surface, is expected to increase with the wind shear

stress in the case of a homogeneous column, which is likely to occur during the night-time. However, in the presence of stratification, which is likely to occur during the day-time, turbulent mixing would result in the reduction of the DO content at the surface, due to the re-suspension of waters with low DO content. The effect of the wind-mixing to make the water column homogeneous is illustrated by Figure 6, which shows clearly that the differences between the DO measured at the distant station (E0) and those measured in the aquaculture net cages decreased with the increased wind shear stress, implying dilution and water exchange in the net cages brought by the wind.

The tendency of the wind shear stress to reduce BOD may be explained by the fact that wind promotes turbulence and water exchange, diluting water and dispersing organic matter, resulting in the reduction of its concentration, and consequently, reducing the pollution and improving the water quality in the fish growing net cages. Based on the relationship between BOD and wind shear stress, wind contributed about 20% to water renewal, hence, the remaining 80% is due to other factors which include the production and consumption of DO in the water column, the fish density and the mesh size of the net cages, as this may restrict water exchange. However,



Figures 6: Relationship of the wind shear stress and the DO differences between measurements at the distant place (E0) and those in the net cages, taken at 8:00 o'clock, in the tilapia aquaculture farm in Nhabavale Lagoon, Chidenguele, during 21 September to 21 October 2014.

considering the fact that the wind effect is continuous and persistent, its cumulative effect in water exchange in the aquaculture system may be enormous. The findings in this study are in agreement with those obtained by Ro and Hunt (2006) who reviewed research on oxygen and other gas transfers from the atmosphere into non-moving, open water bodies, and developed a model for the wind-driven oxygen diffusion from the atmosphere to the water body, based on data published over a period

of 50 years. Their results indicated that wind was the major turbulence agent facilitating gas transfer processes between the interface atmosphere and water. Low wind speed did not significantly influence the intensity of gas transfer. However, the transfer speed increased, even exponentially, with higher wind speeds. The result of the present study is also in agreement with earlier studies by (Banks, 1975; Crusius and Wanninkhof, 2003; EPA, 2012) who in separate studies showed that gas exchange

velocity increases with wind speed.

The low ($r^2 \approx 0.20$) and statically less significant ($p \approx 0.2$) correlation between wind shear stress and DO and BOD may be due to the time lag between the wind force and its effect on the water column. There should be a best time lag between the wind occurrence and its effect in the water column. Studies by Ro and Hunt (2006) found a 12 hour time lag between the winds and the associated state of sea level, whereas Miller (1957) found the best correlation between the wind and the effect of the wind-induced mixing on the vertical distribution of buoyant and sinking of phytoplankton species at a time lag of 10-30 min after the wind blow. In this present study there was a 10 min time lag.

Concluding remarks

This study showed clearly, though with low coefficient of goodness of fit, the relationship between the wind shear stress and DO and BOD. The wind shear stress generates ripples at the surface which enhances the dissolution of oxygen, creates turbulent diffusion which causes vertical mixing and water exchange resulting in improved water quality in aquaculture net cages. The findings of this present study may contribute to the determination of the carrying capacity and further, reiterates the importance of climatic factors in aquaculture systems in natural open water courses.

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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
June 2019

Aims and scope

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

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

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

- Veterinary microbiology, epidemiology
- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
- Wildlife management
- Fisheries and aquaculture development
- Food safety and food hygiene
- One health
- Emerging and re-emerging issues in animal resources
- Biosecurity
- Animal resources trade and value chain
- Socio economics and economics of animal resources development

Language

The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese), by the editors, after acceptance. Full articles submitted in French will also be published in English.

Manuscripts Submission

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

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

To be considered for publication in the BAHPA, any given manuscript must satisfy the following criteria:

- Originality. BAHPA does not accept manuscripts that have already been published elsewhere. However, studies that replicate results that are already in the literature may be considered for publication, as the independent confirmation of results can often be valuable, as can the presentation of a new dataset.
- Audience. Manuscripts submitted must be of broad interest to animal health and production professionals in general, they must capture and hold readers' attention.
- Usefulness. Manuscripts submitted must help researchers, trainers, educators and policy makers in all regions of Africa improve their effectiveness.
- Rigorous methodology. Manuscripts submitted must be based on valid and reliable information, documentation or sound concepts, empirically, logically and theoretically supported.
- Well written to ensure clear and effective presentation of the work and key findings. The BAHPA editorial staff does not copyedit the text of accepted manuscripts, it is therefore important for the work, as presented, to be intelligible. Perfect, stylish language is not essential but it must be clear and unambiguous. If the language of a paper is not clear, Academic Editors should recommend that authors seek independent editorial help before submission of a revision. Poor presentation and language is a justifiable reason for rejection.
- Experiments, statistics, and other analyses performed are described in sufficient detail. The research must have been performed to a technical standard to allow robust conclusions to be drawn from the data. Methods and reagents must also be described in sufficient detail so that another researcher is able to reproduce the experiments described.
- Conclusions are presented in an appropriate fashion and are supported by the data. The results must be interpreted appropriately, such that all conclusions are justified. However, authors may discuss possible explanations for their results as long as these are clearly identified as speculations or hypotheses, rather than as firm conclusions. Inappropriate interpretation of results is a justifiable reason for rejection.
- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.
- Because the guidelines are updated as appropriate, authors should check them again before they submit their articles. Manuscripts submitted for publication will be considered for acceptance on the understanding that they present original work which has not been published or submitted for publication elsewhere and that they are subject to peer review.

Types of contribution

Full papers providing accounts of original work: Research containing significant new findings. The material presented should be original and not have been published elsewhere, except in a preliminary form. Papers will be reviewed by three referees familiar with the subject matter of the paper.

Short Communications: are intended to provide quick publication of highly relevant and interesting information. Manuscripts will be peer reviewed by two reviewers and the Editor.

Review Articles: should cover subjects falling within the scope of the bulletin, which are of active current interest. Papers need not contain original work or ideas. They will be reviewed for completeness, accuracy, style and suitability of content by referees familiar with the subject and the Editor-in-Chief.

Editorial: articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.

Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

Key notes and special calls: The editor will, from time to time, invite selected key figures in the field of animal health and production for key notes on specific topics. Book Reviews: are accepted and should provide an overview of the work's contents and a critique of the work's value. Book reviews should be limited to 1000 words.

Conference Proceedings: Special Issues of the bulletin may be dedicated to publication of proceedings of key meetings/conferences

Obituary articles to honor prominent African scientists that have made significant contribution to animal resources research and development

News and announcements: BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information.

Submission Guidelines

Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

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

repetitive way. Subheadings may be accepted.

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

Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double line spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
6. Place page numbers in the lower right hand corner of your manuscript.
7. Run "the spell check" and "grammar check" on the entire file before submission using either the UK English or French standard.
8. Avoid using abbreviations for the names of concepts. Use ordinary words for variable names – not code names or other abbreviations. Use the same name for a variable throughout your text, tables, figures and appendices. Names of organizations and research instruments may be abbreviated, but give the full name (with abbreviation in brackets) the first time you mention one of these.
9. References should take the following form: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by ' *et al.*'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works. Examples: Abayomi (2000), Agindotan *et al.*, (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1995, 1993), (Kumasi *et al.*, 2001)

The use of reference managing software is encouraged

The authors should be cited in a chronological order by year and then by a or b; in the reference list they should be listed alphabetically.

Please ensure that references in the text exactly match those in the manuscript's reference list. Check each reference in the text to see that you have the complete citation in the reference section of the paper in the desired style. In the references section, references are listed in alphabetical order.

Examples of References

- **Journal Articles:** Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. *Journal of American Science*, 1(2): 14-21.
- **Books:** Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*. London, Cambridge University Press.

- *Chapter in a Book*: Leach J, 1993. Impacts of the Zebra Mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs of Western Lake Erie. In *Zebra Mussels: Biology, Impacts and Control*, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports*: Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings*: Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis*: Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links*: Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from <http://www.lrrd.org/lrrd16/4/cero16020.htm>

Illustrations

Please send the figures as separate files and do not import them into the text file. Put all tables, figures, diagrams and artwork on separate pages. Each figure, table, and bibliographic entry must have a reference in the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". The Editor will place them in the appropriate place in the text of article during the final edit. Tables and figures should be numbered consecutively. Please submit the data for figures in black and white.

Abbreviations, Symbols and Nomenclature

All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names should be italicized in the manuscript.

Ethical guidelines

BAHPA adheres to the below ethical guidelines for publication and research. Experimentation will only be published if such research has been conducted in full accordance with ethical principles. Manuscripts containing experimentations must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

1. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
2. All studies using animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

Revising your article

When you submit a revised version of your article in response to the referees' comments, you must accompany it with a detailed list of the changes made (ignoring typographical errors, but mentioning additional paragraphs, changes to figures, etc) suitable for transmission to the referee. Where changes have been made in response to the referees' remarks it is important to mention this and indicate where they can be found. You may also wish to send in a second copy of your article with the changes marked or underlined.

You should go through the referees' comments and for each comment mention whether you followed their suggestion or whether you disagree and wish to respond to the comment. If a referee has misunderstood a point, it is not necessarily their fault and may have been caused by ambiguity or lack of clarity in your article which needs to be corrected. Some authors copy out each of the referees' comments in turn and include their response immediately after. In other cases responses can be made referring back to the reports. Finally, please make sure that you send your revised article to us and not simply the original version again. This is a common mistake, especially when authors send in their work electronically. Electronic revised articles should contain all text and graphics files needed to generate the revised version, and not just those files that have changed.

By observing these guidelines you will be assisting the referees, who give up their time to review manuscripts. If you prepare your article carefully, this can save valuable time during the publication process.

Appeal of Decision

Authors who wish to appeal the decision on their submitted paper may do so by e-mailing the editorial office with a detailed explanation for why they find reasons to appeal the decision within 14 days.

Proofs

One set of proofs will be sent to the author to be checked for printer's errors and should be returned within three days.

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