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CONTROL OF *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CHICKEN MEAT AND CHICKEN PRODUCTS BY DIPPING IN SOME ORGANIC ACID SOLUTIONS

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CONTRÔLE DE LA *LISTERIA MONOCYTOGENES* ET LE *STAPHYLOCOCCUS AUREUS* DE LA VIANDE DE POULET ET DE PRODUITS DE POULET ISOLÉS PAR LE TREMPAGE DANS CERTAINES SOLUTIONS D'ACIDES ORGANIQUES

Sommaire

Les échantillons de viande fraîche désossée de poulet (140 échantillons de cuisse et 180 de muscle pectoraux) recueillies auprès de différentes boutiques et marchés dans le gouvernorat de Sharkia, en Égypte ont été examinés pour détecter la présence de *L. monocytogenes* et *S. aureus. S. aureus* a été isolé dans 48 (34,3%) échantillons de cuisse comprenant en moyenne 7.8 x 103 ± 2.1 x 102 CFU/gm., et dans 91 (50,6%) de muscle pectoraux avec en moyenne 2.4 x 103 ± 0.7 x 102 CFU/gm). *L. monocytogenes* a été isolée dans 16 (11,4%) échantillons de cuisse de poulet (2.3 x 102 ± 0.7 x 10 CFUgm), et dans 33 (18,3%) échantillons de muscle pectoraux (0.9 x 102 ± 0.2 x 102 CFU/gm). La susceptibilité aux antimicrobiens des micro-organismes testés a montré que la résistance de *S. aureus* aux antibiotiques variait de 12,9% (pour Kanamycine) à 40,3% (pour le chloramphénicol), tandis que celle de *L. monocytogenes* aux antibiotiques a varié de 14,3% (érythromycine) et 100% (Colistine sulfate). Cette étude montre l'importance de *S. aureus* et *L. monocytogenes* comme deux agents pathogènes contaminant la viande de poulet et, par conséquent, la contrôle régulier de la viande de poulet contre ces agents pathogènes est essentiel pour améliorer la qualité des produits livrés aux consommateurs.

Summary

Fresh chicken boneless meat samples were collected from different shops and markets at Sharkia Governorate, Egypt. All samples were examined for the presence of *Listelia monocytogenes* and *S. aureus* by the standard microbiological procedures. *S. aureus* was isolated from 48 (34.3%) samples out of 140 examined thigh muscle samples with the mean count of $7.8 \times 10^3 \pm 2.1 \times 10$ CFU/gm. , and from 91 (50.6%) out of 180 examined breast muscle samples with the mean count of $2.4 \times 103 \pm 0.7 \times 10^2$ CFU/gm. *L. monocytogenes* was isolated from 16 (11.4%) samples out of 140 examined chicken thigh samples with a mean of 2.3 x 102 ± 0.7 x 10 CFU/gm., and from 33 (18.3%) samples out of 180 examined chicken breast samples with a mean count of $0.9 \times 10^2 \pm 0.2 \times 10^2$ CFU/gm. The antimicrobial susceptibility patterns of the tested microorganisms showed that resistance of *S. aureus* for antibiotics ranged from 12.9% *(Kanamycin)* to 40.3% *(Chloramphenicol),* while; that of *L. monocytogenes* for antibiotics varied between 14.3% *(Erythromycin)* and 100% *(Colistin sulphate)*. This study shows the importance of *S. aureus* and *L. monocytogenes* as two risky food-borne pathogens contaminating chicken meat; therefore, regular testing of chicken meat for these pathogens is essential for improving the quality of products supplied to the consumer, controlling the hazards in poultry products and enhancing food safety.

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Introduction

It is well documented that the contamination of food with pathogens is a major public health concern worldwide. Poultry meat contributes substantially to the human diet as an important, low cost source of animal protein. Poultry meat is increasingly used by the growing rural and urban populations[1].

Because of the relatively high frequency of contamination of poultry with pathogenic bacteria, raw poultry products are reported to be responsible for a significant number of cases of human food poisoning. In the absence of hygienic conditions, the birds may be highly exposed to bacterial pathogens. *Staphylococcus aureus,* and *L. monocytogenes* are considered important food-borne pathogens[2].

Although *L. monocytogenes* is responsible for few listeriosis outbreaks, the high mortality rate accompanying infection, its wide distribution in nature and its ability to grow at refrigeration temperature have propelled *L. monocytogenes* into the spotlight among food borne pathogens[3].

Staphylococcus aureus is a significant cause of avian disease and may thus contaminate foods as a result of processed carcasses. While *staphylococci* commonly occur on the skin and nasopharynx of healthy poultry, *S. aureus* which can survive, colonize, and persist at various processing stages in commercial poultry processing plants producing a common food-borne human illness throughout the world[4].

In response to demands from consumers for safer meat and poultry products and implementation of government regulations, numerous studies testing possible interventions have been conducted, particularly over the last 20 years. A wide variety of approaches to sanitize meat and poultry products after harvesting have been developed. Among these approaches is acidification of foods with short-chain organic acids, either by fermentation or by deliberate addition. It is an important and widespread mechanism for controlling food-borne pathogens in a variety of foods. However, a number of studies have demonstrated that *L. monocytogenes* is more acid tolerant than most food-borne pathogens, although the sensitivity of the organism to organic acids varies with the nature of the acid used[5].

The main studies performed on chickens have focused on evaluating their hygienic quality. Unfortunately few studies have assessed the hygienic quality of raw poultry meat particularly its contamination with *L. monocytogenes* pathogen in Egypt[6]. The objectives of the present study was to determine the prevalence of *L. monocytogenes* and *S. aureus* microorganisms in chickens' meats in Sharkia Governorate and to examine the growth inhibition effects of some organic acid solutions (lactic, acetic and citric acids) on these particular pathogens.

Materials and methods

This work was carried out in a Collaboration between Microbiology Department, Faculty of Medicine, Zagazig University, Egypt and Food hygiene department. Animal health research Institute, Zagazig, Egypt. The study included 320 fresh chicken boneless (skin on) meat samples (180 breast and 140 thigh meat samples). The samples were obtained from different shops and markets at Sharkia Governorate, Egypt, collected in sterile polystyrene bags and transferred to the laboratory in an ice

box for testing.

For isolation of *L. monocytogenes,* 25 gm from each chicken sample were blended, added to 225 ml *Listeria* enrichment broth (Oxoid, France) and incubated at 30°C for 48 h. The enrichment culture was streaked on PALCAM selective agar (Oxoid) and incubated at 30°C for 48h[7]. Suspected colonies were identified as being *L. monocytogenes* by Gram stain, motility, V.P., catalase and oxidase tests[8].

For isolation and identification of *S. aureus,* 10 gm from each meat sample were blended and added to 90 ml of 0.1% sterile peptone water. Then, 0.1 ml of the mixture was inoculated into Baird Parker agar plate (Oxoid), incubated at 37^{0}C for 24 - 48 h. Suspected colonies were identified by Gram staining, culture characters and coagulase test[9].

Drug susceptibility testing of *L. monocytogenes* and *S. aureus* isolates was performed on Muller Hinton agar by disc diffusion method using the following antimicrobial discs (Oxoid): ampicillin (10 ug), chloramphenicol (30 ug), Erythromycin (15 ug), Gentamycin (10 ug), Kanamycin (30 ug), methicillin (5 ug) and Rifampin (30 ug) for *S. aureus* and amoxicillin (25 ug), cefadroxil (30 ug), ciprofloxacin (5 ug), colistin sulphate (50 ug), erythromycin (15 ug), tetracycline (30 ug) and streptomycin (10 ug) for *L. monocytogenes.* The results were interpreted according to National Committee for Clinical Laboratory Standards[10].

After isolation and identification of *L. monocytogenes* and *S. aureus*, one isolate of each pathogen was cultivated on tryptic soy broth at 30^{0}C (for *Listeria*) and 37 °C (for *S. aureus*) for 24 h, then centrifuged at 2000 rpm for 15 minutes, washed in phosphate buffer saline and diluted to obtain 108 CFU/ml. Then, 1 ml was inoculated to each meat sample (the sample were confirmed experimentally to be free from any microorganisms). Each of acetic, citric and lactic acids (Sigma, USA) was added in concentrations of 0.5%, 1% and 5% for each acid to meat samples contaminated with *L. monocytogenes* and *S. aureus* for 30 seconds, 1 and 5 minutes. *Listeria* was counted using PALCAM agar and *S. aureus* using Baird Parker agar[10].

Results and Discussion

Table 1: The prevalence and microbial count per gm. of *L. monocytogenes* and *S. aureus* isolates among chicken meat samples.

Microorganism	Chicken thigh (No = 140)			Chicken breast (no = 180)		
	Microbial count Mean ± SD	*No*	%	*Microbial count Mean ± SD*	*No*	%
S. aureus	$7.8 \times 10^3 \pm 2.1 \times 10^2$	48	34.3	$2.4 \times 10^3 \pm 0.7 \times 10^2$	91	50.6
L. monocytogenes	$2.3 \times 10^2 \pm 0.7 \times 10$	16	11.4	$0.9 \times 10^2 \pm 0.2 \times 10^2$	33	18.3

Table 2: The antimicrobial susceptibility patterns of *L. monocytogenes* and *S. aureus* isolates.

Antibiotics	Resistant isolates	
	No	%
S. aureus (No = 139)		
Amplicillin	29	20.9
Chloramphenicol	56	40.3
Erythromycin	34	24.5
Gentamycin	20	14.4
Kanamycin	18	12.9
Rifampin	21	15.1
Methicillin	25	18
L. monocytogenes (No = 49)		
Amoxicillin	32	65.3
Cefadroxil	12	24.5
Ciprofloxacin	17	34.7
Colistin sulphate	49	100
Erythromycin	7	14.3
Tetracycline	30	61.2
Streptomycin	20	40.8

Table 3: The inhibitory effects of organic acids treatment on count of *L. monocytogenes* in chicken meat samples.

Organic acid	Time	Concentration (CFU/gm)		
		0.5%	1%	5%
Acetic acid	0.5 min	3×10^8	3×10^6	2×10^4
	1 min	2.5×10^8	2.5×10^6	1.5×10^2
	5 min	1.5×10^8	1×10^6	1.5×10^1
Citric acid	0.5 min	5×10^8	4×10^7	2×10^5
	1 min	3×10^8	1.5×10^7	3×10^4
	5 min	1×10^8	1×10^5	2×10^3
Lactic acid	0.5 min	3×10^8	3×10^5	2×10^2
	1 min	2×10^8	$2\ 10^4$	1.5×10^1
	5 min	1×10^6	1.5×10^3	0

Table 4: The inhibitory effects of organic acids treatment on count of *S. aureus* in chicken meat samples.

Organic acid	Time	Concentration (CFU/gm)		
		0.5%	1%	5%
Acetic acid	0.5 min	5×10^8	4×10^7	3×10^5
	1 min	3.5×10^8	2.5×10^6	2×10^4
	5 min	1×10^8	1×10^6	1.5×10^2
Citric acid	0.5 min	3×10^8	6×10^7	4×10^6
	1 min	2.5×10^8	4×10^7	3.5×10^4
	5 min	1×10^8	3×10^5	3×10^3
Lactic acid	0.5 min	4×10^8	3×10^6	2×10^4
	1 min	3×10^8	2×10^6	1.5×10^4
	5 min	2×10^6	1×10^4	1×10^3

Because the consumption of poultry meat and poultry meat products show an upward trend, adequate control and inspection in poultry industry are required. An efficacious way of preventing food-borne human diseases is to monitor the microbiological quality of poultry meat and meat products during production, storage and distribution. This work aims to determine the prevalence of *L. monocytogenes* and *S. aureus* microorganisms in chickens' meats in Sharkia Governorate, and to examine the growth inhibition effects of some organic acid solutions (lactic, acetic and citric acids) on these particular pathogens.

In the current study, *S. aureus* was isolated from 48 (34.3%) samples out of 140 examined thigh muscle samples with the mean count of $7.8 \times 10^3 \pm 2.1 \times 10^2$ CFU/gm . Meanwhile, S. aureus was isolated from 91 (50.6%) chicken breast muscle samples out of 180 examined breast muscle with the mean count of $2.4 \times 10^3 \pm 0.7 \times 10^2$ CFU/gm. Almost similar results had been reported in other local studies[11, 12]. The first study detected *S. aureus* in 48% and 32% of breast and thigh muscles of broilers, while; in the second investigation S. aureus was isolated in 38.7% and 51.5% of examined thigh and breast samples respectively. Moreover, in the second study, the mean staphylococcal counts in thigh and breast samples were $8.9 \times 10^3 \pm 3.4 \times 10^2$ CFU/gm. and $2.7 \times 10^3 \pm 1.7 \times 10^2$ CFU/gm. respectively. Lower prevalence was reported in a recent study[13], which detected 20 (10.4%) *S. aureus* isolates from 192 poultry meat samples. The reason of the high incidence of *S. aureus* in the present investigation may be attributed to the poor personal hygiene of the workers and the technique of hand evisceration practiced in the shops as well as infrequent hand washing and lack of sterilization of utensils and working surfaces. With these factors, a high prevalence of bacteria related to human contact should be expected. Such a high level of contamination with *S. aureus* producing heat stable enterotoxins has been

associated with increased risk of staphylococcal food poisoning.

Considering the prevalence of *L. monocytogenes* in chicken meat samples in the present study, it was isolated form 16 (11.4%) samples out of 140 examined chicken thigh muscles samples with the mean of 2.3 x 102 ± 0.7 x 10 CFU/gm., while; it isolated from 33 (18.3%) samples out of 180 examined chicken breast muscles samples with the mean count of 0.9 x 102 ± 0.2 x 10^2 CFU/gm. A previous study in Pakistan3 showed that the prevalence of *L. monocytogenes* was 12.5 % (5 isolates of 40 chicken meat samples) with a predominance 0 serotype 1 and a less proportion of serotype 4. However, both the serotype 1 and 4 are reported to be pathogenic to man and animals[14]. An European study reported that the prevalence of *L. monocytogenes* among chicken meat samples were 16% and 10% in France and Belgium respectively[15]. However, higher prevalence (48.2 %) in Norway was reported[16]. To our knowledge, local studies concerning the prevalence of *L. monocytogenes* in poultry meat are scanty, a recent Egyptian study recorded 17% overall incidence of *L. monocytogenes* in local poultry meat[17] and another one reported 33% in frozen poultry meat samples[18]. The frequent occurrence of *L. monocytogenes* in poultry meat may pose a potential risk for consumers causing from mild flu-like sickness to sever manifestations as meningoencephalitis and septic infections. One retrospective case-control study undertaken in 6 states in the United States suggested that approximately 20% of the 1600 annual cases of *L. monocytogenes* were likely to have resulted from consuming contaminated undercooked chicken[19].

Nowadays, antibiotics are excessively used in veterinary field and poultry farms for prophylactic and therapeutic options as well as feed additives as growth promoters. This seems to be important risk factors for the acquisition and spread of antimicrobial resistance via selective pressure mechanisms. In this work, the highest resistance of *S. aureus* isolates was to chloramphenicol (40.3%), followed by erythromycin (24.5%) ampicillin (20.9%) and methicillin (18%). It had been reported that *S. aureus* associated with poultry processing are also notorious as reservoir organisms of multiple antibiotic resistance traits[20]. Moreover, there exists ample evidence demonstrating that clonal spread has significantly contributed to the dissemination of multi-resistant *staphylococci* such as methicillin-resistant *S. aureus* in clinical settings[21]. In the current study, the highest sensitivity of *staphylococcal* isolates was to kanamycin (12.9%) and gentamycin (14.4%). So, these antimicrobial agents could be considered the antibiotics of choice. On the other hand, the antimicrobial susceptibility patterns of *L. monocytogenes* isolates in this work showed higher resistance levels. All isolates were resistant to colistin sulphate, while; the resistances to amoxicillin, tetracycline, streptomycin and ciprofloxacin were 65.3%, 61.2%, 40.8% and 34.7% respectively. Similar results had been previously reported[22]. These findings indicated a continuous and increasing incidence of antibiotic resistance in *Listeria* species which might be attributed to the extensive use of antibiotics both in humans and veterinary field. It had been reported that *Listeria* has multiple plasmids encoding antimicrobial resistance to many antimicrobials as

chloramphenicol, erythromycin, tetracycline and streptomycin. In addition another study suggested that resistance to tetracycline may be useful as an easily recognized epidemiological marker for *L. monocytogenes*[23].

In the present investigation, the mean counts of *L. monocytogenes* inoculated in chicken meat samples decreased as the concentration and time of exposure to organic acids increased. The more marked inhibitory effect was observed with lactic acid, followed by acetic and citric acids. A previous study showed that the maximum reduction for *L. monocytogenes* growth was obtained for meat samples dipped in 2% lactic acid for 2 minutes, followed by citric acid with the same concentration[24]. In addition, the effect of lactic acid on the growth and survival of *L. monocytogenes* in crayfish at 0.5%, 1% and 2% concentrations was studied[25] this investigation found that the count decreased steadily with 2% lactic acid. These results signify the importance of choosing an adequate concentration of organic acids used for the decontamination of meat samples as low concentration might promote the growth of *L. monocytogenes.*

The inhibitory action of lactic acid is produced by penetrating the cell membrane, releasing a proton and acidifying the cellular cytoplasm. The inhibitory action of acetic acid is mediated through utilization of electrochemical ingredient of the cell membrane as well as denaturating protein inside the cell but the action of citric acid is partially due to its ability to chelate divalent cations[26].

Considering the inhibitory effect of the same organic acids treatment on *S. aureus* counts in this study, the isolates behaved similarly, but not as dramatically, to *L. monoctogenes.* An investigation studied the inhibitory effects of organic acids on *L. monocytogenes* and *S. aureus* and reported less marked inhibitory effects of organic acids on *S. aureus* than those on the another examined microorganism[27]. Furthermore, the last study showed that the addition of ethylene diamine tetra-acetic acid (EDTA) to the organic acids had a synergistic effect on the both examined organisms. *Staphylococcal* growth and their enterotoxin production in relation to exposure to six organic acids (lactic, citric, ascorbic, acitic, pyruvic and propionic acids) were studied[28] this investigation showed that the more inhibitory acid for *Staphylococcal* growth was lactic acid followed by pyruvic acid, while; the others were equally effective. Moreover, the mentioned study showed that the lactic acid was also very inhibitory to enterotoxin synthesis, but the effect on this parameter of acetic and citric acids was almost nil and enterotoxin B was the more resistant to acid inactivation.

In conclusion, our study signifies the importance of *S. aureus* and *L. monocytogenes* as two risky food-borne pathogens contaminating chicken meat. In addition, regular testing of chicken meat for these pathogens is essential for improving the quality of products supplied to the consumer, controlling the hazards in poultry products and enhancing food safety. Lactic acid may be a more appropriate choice for food treatment as preservative and decontaminating agent since the lack of acute and chronic toxicity of this compound.

Further studies are recommended to identify *L. monocytogenes* serotypes and studying the inhibitory effects of organic acids on *Staphylococcal enterotoxins* in chicken meat.

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EVALUATION OF THE CHEMOTHERAPEUTIC AND PROPHYLACTIC EFFICACY OF KELAMEDIUM® IN BOVINE TRYPANOSOMOSIS

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EVALUATION DE L'EFFICACITE CHIMIOTHERAPEUTIQUES ET PROPHYLACTIQUE DU KELAMEDIUM® POUR LA TRYPANOSOMIASE BOVINE

Sommaire

L'efficacité chimiothérapeutique et chimio-prophylactique de la Kelamedium®, un isométamidium, a été évaluée dans cinq fermes laitières de taille moyenne et deux troupeaux traditionnels ayant 275 et 100 animaux (âgés de 6 mois au moins) respectivement et 316 animaux provenant de 42 troupeaux laitiers. Les animaux ont été examinés cliniquement pour les signes de trypanosomose et les haemoparasites dépistés par frottis sanguins et micro-centrifugation. Les animaux positifs sur des frottis sanguins ont été classés comme «cas» et ont été traités avec une dose curative (1 mg/kg poids vif) de Kelamidium® et surveillés pour la récupération parasitologique au troisième, sixième et neuvième jours. Sur un total de six «cas» détectés et traites, cinq qui ont montré une guérison parasitogique et clinique au 3ème et 9ème jours respectivement, tandis qu'un animal est mort. Les animaux négatifs sur les frottis sanguins ont été assignés au hasard à deux groupes de traitement: un groupe recevant une dose prophylactique (0,5 mg/kg) de Kelamidium ® et l'autre dit «de contrôle» étant traité avec la même dose de Samorin®. Ces animaux ont ensuite été surveillés cliniquement et re-dépistés au 60ème et 90ème jours après intervention pour détecter les re-infections. Le Kelamidium® a fait preuve d'une efficacité prophylactique de l'ordre de 99,4%, comparée (p>0,05) à celle de Samorin® (99,7%). Ces résultats montrent donc que le Kelamidium® offre une alternative au Samorin®.

Mots-clés: Trypanosomose, kelamedium, bovins, haemoparasites chimiothérapeutiques, chimio-prophylactique.

Summary

Chemotherapeutic and chemo-prophylactic efficacy of Kelamedium® an isometamidium was evaluated in five medium dairy farms and two traditional herds with a number of animals above 6 months age being 275 and 100 respectively. The trial also involved 42 smallholder dairy herds with 316 animals. The animals were clinically examined for trypanosomosis signs then screened for haemoparasites using blood smears and micro-centrifugation methods. Positive animals on smears were classified as "cases" and were treated with a curative dose (1 mg/kg bodyweight) of Kelamidium® and monitored for parasitological recovery on days three, six and nine. A total of six "cases" were encountered and five which were treated with Kelamidium® showed parasitogical and clinical recovery by days 3 and 9 respectively, whereas one animal died. Smear negative animals were randomly assigned to two treatment groups; with one group receiving a prophylactic dose (0.5 mg/kg) of Kelamidium® and the controls being treated with the same dose of Samorin®. These animals were then monitored clinically and re-screened on days 60 and 90-post intervention for re-infections. Kelamidium® showed a prophylactic efficacy of 99.4% and this was comparable (p>0.05) to that of Samorin® (99.7%). These results thus show that Kelamidium® offers an alternative choice to Samorin®.

Key words: Trypanosomosis, kelamedium, cattle, haemoparasites chemotherapeutic, chemo-prophylactic*

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Introduction

Bovine trypanosomosis is a major animal disease constraint to livestock production in sub-Saharan Africa. Over 40 million of Africa's cattle populations are kept in tsetse-infested areas, which account for 37% of the continent[1]. In Tanzania, tsetse-borne diseases, and in particular bovine trypanosomosis is one of the two most important diseases that are responsible for reduced livestock productivity and together with tick-borne diseases they are responsible for 75% of the morbidities and mortalities in cattle[2]. Trypanosomosis, which takes the form of an acute to chronic status, is normally characterized by fever, anaemia and loss of productivity; with cattle being the most susceptible domestic animal to *T. congolense, T. vivax* and *T. brucei* infections[3].

Successful raising of cattle in trypanosomosis risk areas in Africa has often largely depended on the use of chemotherapy or chemoprophylaxis and the control of tsetse challenge using acaricides, traps, bush clearing and targets impregnated with insecticides. The complications related to the requirement for natural geographical barriers have limited successes related to the use of the sterile male insect release method as an alternative method. This is explained by its success story in Zanzibar islands alone in Tanzania and not on the Tanzania mainland. Tanzania just like any other country in East and Central Africa is largely dependent on chemotherapy and chemoprophylaxis as the mainstay of controlling the disease. In deed, isometamedium (Samorin®), a drug that has both curative and prophylactic effects against *T. vivax, T. congolense* and *T. brucei* remains to be the most commonly and widely used drug by traditional livestock keepers and those in the small, medium and large-scale dairy sub-sector in Tanzania. As such, the control of the disease in the country will, for unforeseeable future, continue to rely on the use of chemo-prophylactic drugs.

Despite the fact that chemotherapy has always been the most dependable means of control of this economically important disease in Africa, development of new anti-trypanosomal drugs has been more or less static over decades probably due to lack of interest by the pharmaceutical industry to invest into research and development of anti-trypanosomal drugs. However, the situation has changed in the recent past and this is exemplified by the production of Kelamidium® (Isometamidium) by Kela (Hoogstraten Laboratories Belgium). The new product has been manufactured in an effort to widen the scope of trypanocidal drugs in the African market. Kelamidium® is a new trypanocidal product that requires to be tested under Tanzanian condition in order to evaluate its performance. The purpose of this study was therefore to establish the therapeutic and prophylactic efficiency of Kelamedium® in natural infections in cattle.

Materials and methods

Drugs

Kelamidium® kindly provided by Kela (Hoogstraten Laboratories Belgium) and Samorin® purchased from the market were used in this field trial.

Study areas, farms and animals

The field trial on the efficacy of

Kelamidium® for the treatment and control of trypanosomosis was conducted in the urban and peri-urban areas of Morogoro municipality, using smallholder and medium-scale dairy farms. The five medium scale dairy farms (Kingolwira, Lutheran Junior Seminary, Shem, ARU Otitis and Mwilunga), which were purposefully selected, had a total of 275 animals, which were above six months of age. Their choice was purposeful, as there were few farms within the peri-urban areas that could warrant effective use of sampling frames. Two traditional herds (Nasar and Ole) with total of 100 animals above six months of age were also included in the study. In order to mainstream smallholder farms which were already involved in the use of Samorin® for the control of the disease into the study, farms which were being served by two private clinics (KV and SASI) were selected for this study. This permitted the selection of 42 smallholder farms (with a total of 316 animals) which were due for Samorin® inoculations in March 2006. In both smallholder and medium scale sub sectors, the majority of the animals were crosses of Friesian (38.5%), Aryshire (35.3%) or Jersey (1.6%) with local breeds and the rest were local breeds i.e. boran (17.1%) and Tanzania short horned zebu (7.5%). Of the screened animals, 78.3% were female animals and 21.7% were bulls.

Intervention

During the farm visit, herd and animal information about type of animals (i.e. breed, sex and age); use of Samorin® and acaricides for ticks and flies; grazing systems; number of animals in the farm and the farm location was recorded. General health status of the animals was assessed, but emphasis was on animals, which showed signs of ill health. Such animals were thoroughly examined for diagnosis of the disease involved. This was followed by screening all the 375 animals belonging to the five medium scale dairy farms and two traditional herds and, 316 animals in the small-scale dairy herds for hemoparasites. In order to achieve this, briefly, blood smears were collected from the ear veins; air-dried, fixed with absolute ethanol and well packed in slide racks before shipment to the laboratory at SUA. Smears were then stained with 10% Giemsa solution for 30 minutes, washed with tap water and air-dried before examination at x100 magnification with oil immersion. Ten fields in each smear were examined in order to establish whether the smear was negative or positive and quantify the number of parasites (parasitemia level). Any animal with trypanosomal parasites in the blood smear, with or without relevant clinical features, was considered as a "case". Speciation of the parasites by morphology was also undertaken.

Evaluation of the therapeutic performance of Kelamidium®

Although, it was planned that all identified "cases" would have been split into treatment two groups to allow comparative evaluation of performance of Samorin® and Kelamidium®, the few cases that were picked in this study allowed only the use of Kelamidium® using a curative dose rate of 1 mg/kg bodyweight. Nonetheless, these few 'cases' provided clues regarding the therapeutic efficacy of Kelamidium® and thus permitted comparison with the documented therapeutic performance of Samorin®. Briefly, after collecting blood samples for determination of PCV values,

Table 1: Percentage smear positivity for trypanosomiasis during the 90-day observation period.

Visit day	Number of animals	Positive cases (%)	Trypanosome spp (%)		
			T. congolense	*T. vivax*	*T. brucei*
0	691	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)
60	653	3 (0.5)	0 (0.0)	1 (0.2)	2 (0.3)
90	590	2 (0.3)	0 (0.0)	2 (0.3)	0 (0.0)
Total		6 (0.9)	1 (0.1)	3 (0.5)	2 (0.3)

the animals were treated with Kelamidium® and then they were clinically examined and screened for hemoparasites on days 3, 6, 9 in order to determine the clinical status and parasitological recovery rates.

Evaluation of the prophylactic performance of Kelamidium®

All smear negative animals in each study farm were then randomly assigned to two treatment groups; with animals in one group being injected with a prophylactic dose of Kelamidium® and the control animals received the same dose of Samorin®. For both Kelamidium® and Samorin®, a dose of 0.5mg/kg body weight was used intramuscularly in the neck muscles. All study animals were then clinically examined (through quick herd assessment, with emphasis being placed on those with clinical signs) and screening for parasites on days 60 and 90 post-intervention. Animals that were found to be smear positive for trypanosomes between (as picked by local field officers as part of their routine engagement in the farms) and during the farm visits, regardless of the drug used in the trail, were immediately treated with Kelamidium® using the above indicated curative dose.

Results

The results of the evaluation of the chemotherapeutic and prophylactic efficacy of Kelamedium ® on bovine trypanosomosis are summarized in Tables 1 and 2. Tables 3 and 4 show the number of "cases" in respect of various farm and animal factors. Briefly a total of 691, 650 and 590 cattle were screened for trypanosomosis on days 0, 60 and 90 respectively. Specific infections rates based on smear positivity for trypanosomosis were 0.1%, 0.5% and 0.3% for days 0, 60 and 90, respectively (Table 1). Thus, in this trial, Kelamedium ® showed prophylactic efficacy of 99.4% against trypanosomosis in cattle for 90 days post treatment, whereas that of Samorin® was 99.7%. The difference in effectiveness in preventing trypanosomosis between the two drugs was statistically not significant (P>0.05).

Table 2: Number of "cases" and *Trypanosoma* species in different treatment groups

Follow up days	Drug	Number of animals	Cases occurrence (%)	Tryp spp (%)		
				T. congolense	*T. vivax*	*T. brucei*
60	Kela	349	2 (0.6)	0 (0.0)	1 (0.3)	1 (0.3)
	Samorin	304	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.3)
90	Kela	319	2 (0.6)	0 (0.0)	2 (0.6)	0 (0.0)
	Samorin	271	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 3: Percentage frequency of *trypanosomosis* “cases” based on animal factors.

Variable	Description	Test day	Number examined	Number infected (%)
Sex	Female	0	541	1(0.2)
		60	520	3(0.6)
		90	479	2(0.4)
	Male	0	150	0(0.0)
		60	153	0(0.0)
		90	111	0(0.0)
Breed	Aryshire cross	0	244	0(0.0)
		60	229	1(0.4)
		90	205	1(0.5)
	Friesian cross	0	266	0(0.0)
		60	255	2(0.8)
		90	227	1(0.4)
	Jersey cross	0	11	0(0.0)
		60	10	0(0.0)
		90	8	0(0.0)
	Boran	0	118	0(0.0)
		60	107	0(0.0)
		90	98	0(0.0)
	Zebu	0	52	1(1.9)
		60	52	0(0.0)
		90	52	0(0.0)

Table 4: Percentage frequency of *trypanosomosis* “cases” based on farm factors.

Variable	Description	Test day	Number examined	Number infected (%)
Grazing systems	Extensive (Traditional herds)	0	100	1 (1.0)
		60	100	0 (0.0)
		90	100	0 (0.0)
	Stall feeding	0	67	0 (0.0)
		60	65	0 (0.0)
		90	59	0 (0.0)
	Grazing combined with a “cut and carry” system of feeding	0	524	0 (0.0)
		60	488	3 (0.6)
		90	431	2 (0.5)
Farm size	Small	0	375	0 (0.0)
		60	296	1 (0.3)
		90	275	1 (0.4)
	Medium	0	316	1 (0.6)
		60	357	2 (0.6)
		90	315	1 (0.4)
Acaricide use	Uses	0	591	0 (0.0)
		60	553	3 (0.5)
		90	490	2 (0.4)
	No use	0	100	1 (1.0)
		60	100	0 (0.0)
		90	100	0 (0.0)
Location	North/eastern	0	303	1 (0.3)
		60	285	3 (1.1)
		90	278	2 (0.7)
	South/western	0	388	0 (0.0)
		60	368	0 (0.0)
		90	312	0 (0.0)

A total of six "cases" were encountered in the study farms. One "case" was encountered on day 0 (prior to treatment of animals), whereas one and two animals were detected in the Samorin® and Kelamedium ® -treated animals respectively on day 60. Two additional "cases "were detected only in the Kelamedium ® treated animals on day 90. Five of the "cases" (83.3%) were exotic animals (crossbreds) in the medium scale farms and only one (16.7%) belonged to the traditional herd (Table 3). Five out of the six "cases" were found in animals which were grazed and at times stall fed and the location of the farms was also found to be a risk factor of infection as all the "cases" were diagnosed from the farms situated in the north/eastern part of Morogoro, which is known to have high tsetse fly challenge (Table 4).

The six "cases" were infected by *Trypanosoma vivax* (3), *Trypanosoma brucei* (2) and *Trypanosoma congolense* (1). The infected animals had the mean rectal temperature of 39.10C±1.03, with the mean number of parasite and PCV being 15±21.7 and 24.8% ±7.92, respectively. Interestingly, one animal had parasitaemia level of up to 58 trypanosomes in 10 fields and the PCV was 16%. Out of the six "cases" that were treated with the Kelamedium® at the dose of 1 mg/kg body weight, five (83.3%) showed parasitological recovery by day 3 whereas clinical improvement was evident by day 9. Only one (16.7%) died following treatment and this was the animal, which was recumbent and had poor body condition at the time of treatment.

Discussion

This trial was set up to evaluate the prophylactic efficacy of new product (Kelamedium®) of isometamedium against natural trypanosomal infections in cattle for a period of 90 days. This new formulation, when used at the recommended dose of 0.5mg/kg intramuscularly, showed a prophylactic efficacy of 99.4% against trypanosomosis. The high level of protection of animals by Kelamedium® during the 90-day post intervention observation period was comparable ($p>0.05$) to that offered by Samorin®, which has been in the African market since 1961 and which protects animals for three4 to five months5, 6 depending on the vector density, species and strains of trypanosomes and the dose of the drug. The results of this study therefore indicate that Kelamedium® is as effective as Samorin® and that the drug offers an alternative choice for use in the protection of animals against this important disease.

The occurrences of four "cases" (1.2%) in the animals treated with Kelamedium® could suggest possibilities for high trypanosomal challenge; poor body condition of the animals leading to poor re-absorption of drugs from the injection sites and resistance to isometamedium that may be attributable to antigenic variation of trypanosomes or repeated usage of the drug at a relatively low dose. In deed, Peregrine et al (1988) reported relapses of *T. vivax* and *T. congolense* and noted much greater levels of resistance to prophylactic activity of isometamedium by these two species. Resistance has, also been reported by Eisler[7] involving *T. vivax,* which is the most prevalent trypanosome in East Africa. However, the observation that three "cases" belonged to one farm where the body condition of most of the animals was poor, suggests that debilitation and hence compromised body immunity may have led

to the occurrence of such cases.

When Kelamedium® was used for treatment of the six "cases" at a dose of 1 mg/kg, 83.3% of the animals recovered and the one which died following treatment was an advanced case of trypanosomosis. This was the case, which was already recumbent at the time of treatment; had poor body condition and slight subnormal rectal temperature (37.8^{0}C) and was anorexic for the previous four days. This animal possibly died because the diagnosis and treatment were very much delayed. It is obvious that the therapeutic evaluation of the drug in this study was limited by the number of "cases" (6) encountered during the 90 days of monitoring the study farms and animals. Nevertheless, the drug was capable of clearing parasitaemia even in situations of high parasitaemia levels (up to 58 parasites in 10 fields) by day 3-post treatment as it applies to diminazene aceturate8. This shows that Kelamedium® can offer an alternative to dimenazene aceturate in the treatment of trypanosomosis in cattle.

The study has also shown that trypanosomosis exists in the urban and periurban areas of Morogoro municipality but at a low rate (0.9%). The low smear positive rate is probably due to the observation that up to 77.1% of the farms were routinely (every three months) using samorin for prophylaxis and that many (85%) were also using acaricides such as pyrethroids that have fly repellant characteristics. For instance, in the study conducted by Msolla9, a 93% reduction of tsetse flies in Melela was reported following the use of alpha-cypermethrin 10% preparation. However, it is possible that the low rate of infection is due to the use of less sensitive diagnostic tools (blood smears and micro-centrifugation).

In conclusion, the prophylactic performance of Kelamedium®, which was comparable to that of Samorin® during the 90 days of observation, suggests that this new formulation is capable of protecting animals against natural infections (trypanosomosis) and it can thus be adopted for use in order to reduce the economic effects of the disease. In addition, the high therapeutic performance of this drug, albeit the few animals that were mainstreamed in the clinical intervention trial, indicates that Kelamedium®, can also serve as a dependable alternative drug to dimenazene aceturate in clinical cases of trypanosomosis. However, more studies, using adequate number of cases, need to be carried out in order to ascertain the therapeutic efficacy of the drug.

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PARASITOLOGICAL PREVALENCE OF BOVINE TRYPANOSOMOSIS IN FARO AND DEO DIVISION CAMEROON, TEN YEARS AFTER THE TSETSE ERADICATION CAMPAIGN

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PREVALENCE PARASITOLOGUE DE LA TRYPANOSOMOSE BOVINE DANS LE DÉPARTEMENT DU FARO ET DEO AU CAMEROUN, 10 ANS APRES UNE CAMPAGNE D'ÉRADICATION DE LA MOUCHE TSÉ-TSÉ

Sommaire

Dix ans après la campagne d'éradication des glossines, une enquête transversale visant à déterminer la prévalence de la trypanosomose et l'identification des espèces de trypanosome en présence a été effectuée dans le département du Faro & Deo, plateau de l'Adamaoua, Nord Cameroun. Au total, 302 bovins adultes ont été examinés dans vingt localités différentes appartenant soit à la zone assainie, tampon ou infestée. Les techniques de diagnostic utilisées consistaient en un examen de la couche leucocytaire sur fond noir, un examen d'un frottis mince colore et une évaluation de l'hématocrite. La prévalence totale de la trypanosomose bovine était de 14,6%, mais variait significativement par zone. Elle était élevée en zone infestée 35.1% et basse dans la zone assainie (4.3%) et tampon (5.3%); et aucune différence significative (P = 0.051 > 0.05) n'été observé entre la zone tampon et le plateau. Parmi les animaux positifs, 43.2%, 13.6%, 9.1%, 13.6% et 20.5% étaient causes par *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma brucei,* et des infections mixtes (*T. congolense* et *T. vivax*) et (*T. congolense, T. brucei* et *T. vivax*) respectivement. L'hématocrite des animaux positifs et négatifs allait de 16 à 40 pour cent et de 15 à 46 pour cent, respectivement. L'hématocrite moyen des animaux négatifs (29,6±5.7%) était significativement plus élevé que l'hématocrite moyen des animaux positifs (26,9±6.4%) (P = 0.003 < 0.05). Vu le risque de trypanosomose sur le plateau, une stratégie de contrôle appropriée est fortement recommandée.

Mots-clé: Prévalence, trypanosomose, bovin, plateau de l'Adamaoua, Cameroun

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Summary

Ten years after the tsetse eradication campaign, a cross sectional survey to determine the prevalence of trypanosomosis and identifying the species of trypanosomes infecting cattle was conducted in the Adamaoua plateau, North Cameroon. A total of 302 adult cattle were examined at twenty different localities. Field examination of the buffy coat, stained thin blood film examination and packed cell volume (PCV) evaluation were the diagnostic techniques used. The overall prevalence of bovine trypanosomosis was 14.6%. However, the prevalence differed significantly between zones. It was higher in the valley (35.1%) and lower in the buffer zone and the plateau (5.3% and 4.3% respectively); no significant difference (P = 0.051) was found between the buffer zone and plateau. Among the positive animals, 43.2%, 13.6%, 9.1%, 13.6% and 20.5% were from *Trypanosoma congolense, T. vivax , T. brucei* and mixed infection (*T. congolense* & *T. vivax*) and (*T. congolense, T. brucei* & *T. vivax*), respectively. The mean PCV of positive and negative animals ranged between 16 - 40 % and 15 - 46%, respectively. The mean PCV of negative animals (29.6±5.7%) was significantly higher than the mean PCV of positive animals (26.9±6.4%) (P < 0.05). Because of the risk of trypanosomosis on the plateau, appropriate control strategies need to be put in place urgently.

Keywords: Prevalence, trypanosomosis, cattle, Adamaoua plateau, Cameroon

1. Introduction

The tsetse fly *(Glossina spp.)* is responsible for the transmission of protozoan parasites of the genus Trypanosoma, which causes sleeping sickness in humans and *"nagana"* in cattle. In Africa, the total area affected by tsetse flies is larger than the USA and thirty-seven countries[1] are concerned. Some of the measures to control tsetse include "pour-on"[2,3], mass trapping[4,5,6], chemical insecticide impregnated traps or targets[7,8] and sterile insect technique (SIT)[9].

In 1975, the government of Cameroon initiated a project on tsetse eradication by extensive aerial sprays; a large grazing area of Adamaoua plateau was treated. More than 44% (32,000 km^2) of Adamaoua plateau have been treated in 18 years (1976-1994)[10]. To prevent massive reinvasion of tsetse flies from the valley a barrier consisting of targets and traps was put in place by the special tsetse eradication unit (Mission Spéciale d'Eradication des Glossines, MSEG) after aerial spraying. However, bush fires destroyed most of the targets and traps soon after deployment in 1994. Thereafter a program of insecticide-treatments of cattle in the buffer zone replaced the barrier. All the herds grazing in the buffer zone were to be impregnated with insecticide products (pour-on) by breeders. These *"herd traps"* would constitute a virtual boundary against the possible progress of the tsetse fly and would protect the whole livestock situated behind[11,12]. Around 34.2% of the national livestock are also found in this region[13]. The environmental conditions are very suitable for intensive zebu rearing. Three cattle's breeds are reared in the plateau: Goudali (Peuhl de l'Adamaoua), Mbororo Akou (White Fulani) and Mbororo Djafoun (Red Fulani).

Insecticide-treated cattle "herds traps" associated with screen devices and insecticide traps have been used with more or less success in Africa[14,15,16]. The tsetse fly have been controlled successfully using the insecticide impregnated animals[17,18,8,19,20,2,21] but the were also some

failures[22,23,24].

Among the existing disease problems, trypanosomosis is considered as the major livestock production constraint in the area. Trypanosomosis and its associated problems are reported to be very serious in the area in which the present study was conducted[25]. Optimist previsions[26] in 2004, estimated that probably 50% of Adamaoua plateau is cleared from *Glossina spp*.

Before embarking on control or an intervention scheme, epidemiological surveys need to be undertaken to determine the extent of the problem using available diagnostic methods[27]. The present study was undertaken in December 2003 with the main objectives of determining the prevalence of bovine trypanosomosis in the tsetse free area (plateau), the buffer zone and the tsetse infested valley and identifying the species of trypanosomes infecting cattle on the Adamaoua plateau in Cameroon 10 years after the end of the tsetse control interventions.

2. Materials and methods

2.1 Study site

The survey was conducted in Faro & Déo division in the Adamaoua Province of Cameroon. Faro & Déo division is located between latitudes 7^0 and 8^0 North and longitude 12^0 and 13^0 East and covers 11,000 km². At the end of the tsetse eradication campaigns in 1994 the territory was divided in the following three zones situated from south to north (Fig.2). The selected study sites were not previously subjected to intensive investigations on trypanosomosis.

2.1.1 The plateau (tsetse free area)

Constitute the tsetse-free area; it is located between 1,000 and 1,100m a.s.l. with mean temperature 20° C and 40-60% RH. The annual rainfall is 1,800mm and the rainy season lasts from March/April to October. The high annual rain is recorded between June and September. The Adamaoua plateau is covers with savannah consisting of more than 90% of *Daniellia olivert* and *Lophira lanceolata*[28]. This formation, much frequented by the cattle raisers is densest woody by Monotes kerstingii and Uapaca togoensis[29]. The plateau carries more livestock and most of tsetse flies *(G. m. submorsitans)*[12] were cleared from this zone in 1994.

2.1.2 Buffer zone

This zone was identified by the MSEG to act as a barrier to tsetse invasion from the valley to the Adamaoua plateau[12] and is situated in the sub-humid zone between 900 and 1,000m a.s.l., the climate is classified as high-altitude with an average annual rainfall of 1,400 mm. The most frequently occurring vegetation tree is *Isoberlina spp*.[29]. *Daniellia olivert; Lophira lanceolata*[28] and *Sporobolus africanus*[29] are the mainly savannah vegetation formation and land is little used for agriculture.

2.1.3 The infested valley

The plain of Koutine beginning at the bottom of the plateau of Sadec and is an agricultural zone where the cattle keepers of the plateau pass the dry season (transhumance). Is situated between 487m and 1,800m; the grassland vegetation *Sporobolus africanus* occupies the summit of *"Guemfalabo"* mountain. This plant species is preferred grazed by Mbororo cattle[30]. The Koutine plain includes the Faro National Park (Cameroon) and Gashaka National Park (Nigeria). No tsetse control activity has ever been conducted in the

Table 1: Mean parasitological prevalence of trypanosomosis detected by zones and trypanosome infection and mean PCV values of sampling bovine.

Zones				Parasitological prevalence							Mean PCV	
	Numbers of selected localities	Bovines*	Numbers of animals tested	Total numbers of positives animals	Prevalence (%)	*T. congolence*	*T. vivax*	*T. brucei*	*T. congolense* & *T. vivax*	*T. congolense* & *T. vivax* & *T brucei*	Positive animals (n=44)	Negative animals (n=258)
Buffer zone	5	9,693	113	6	5.3	3 (50%)†	-	1 (16.7%)	1 (16.7%)	1 (16.7%)	31.8±6.7	31.5±5.1
Plateau	11	59,647	92	4	4.3	2 (50%)	1 (25%)	1 (25%)	-	-	29.5±6.4	29.5±5.7
Infested valley	4	5,219	97	34	35	14 (41.2%)	5 (14.7%)	2 (5.9%)	5 (14.7%)	8 (23.5%)	25.7±6	26.5±5.4
Total	20	74,559	302	44	14.6	19 (43.2%)	6 (13.6%)	4 (9.1%)	6 (13.6%)	9 (20.5%)	26.9±6.4	29.6±5.7

* Census data 2003 (DDEPIA/FD, 2003); †: numbers in parenthesis indicate the relative proportion of *T. vivax*, *T. vivax* and *T. brucei* infection at each zone

Table 2: Mean parasitological prevalence of trypanosomosis detected by races and mean PCV and weight of examined bovines

Race	Goudali (Peuhl of Adamaoua)				White foulani (Akou)				Red foulani (Djafoun)				Charolais (Goudali x Charolais)			
	n	Prevalence (%)	Mean PCV	Mean weight	n	Prevalence (%)	Mean PCV	Mean weight	n	Prevalence (%)	Mean PCV	Mean weight	n	Prevalence (%)	Mean PCV	Mean weight
Buffer zone	56	5.4 (2/56)	31.6±5	339.9±71	40	7.5 (3/40)	32.4±5	311.7±73	17	0	28.9±4.7	343.6±95	0	-		
Plateau	38	5.3 (2/38)	29.3±5.6	282.6±80	38	5.3 (2/38)	30.6±5.7	285.5±5	10	0	27.8±6	235±84	6	0	26.7±5	321±51
Infested valley	1	0	18	286	68	36.8 (25/68)	25.2±5	256.1±58	28	32.1 (9/28)	29±6	255.4±63	0	-		
Total	95	5.3 (5/95)	146	55	6											

valley.

2.2 Experimental design

A stratified sampling strategy was used which involved the random selection of twenty locations (Table 1) into three geographical zones (plateau, buffer zone and infested valley) based on partition put in place by the tsetse eradication unit (Mission Spéciale d'Eradication des *Glossines*) and altitude. From each zone, three localities were selected randomly and one, two and eight more were added respectively to the tsetse-infested valley, the buffer zone and the plateau as a contingency for the proportion of cattle that are kept there and the vastness of the area. Therefore 302 cattle from 39 herds were sampled from a population of 74,559 in the division.

2.3 Sample collection and diagnostic techniques

Examination of the buffy coat and stained thin blood films were the diagnostic test used. Thin blood films were prepared from a drop of blood obtained by venepunture of visible veins using a lancet. Blood films were fixed on site with methanol and stained with 10% Giemsa solution at the laboratory. Whole blood was evacuated from an ear veins using heparinized micro-haematocrit centrifuge capillary tubes. The capillary tubes were sealed with *"cristaseal"* (Hawksley, Lancing, UK) and centrifuged on site in a microhaematocrit centrifuge for 5 min at 7500 rpm. A portable regular gasohol generator of 10 kv was used as power source in the field. After centrifugation packed cell volume (PCV) of each sample was determined. Animals with PCV readings below 25% were considered as anaemic31. The haematocrit tubes were cut a few millimeters below the junction of the buffy coat-plasma levels, and the erythrocytes, buffy coat and plasma of each specimen was expressed onto a microscope slide and examined with a phase-contrast microscopic with an x 40 objective lens for the presence of mobile trypanosomes[32]. When the sample was found positive for a trypanosome, a thin smear was prepared, fixed, stained with Giemsa and examined with the 100X (oil immersion) objective lens for species identification.

3. Results

3.1 Parasitological prevalence

A total of 302 cattle were examined. Trypanosomes were detected in 44 cattle (14.6%) the trypanosome species involved were *T. congolense* 43.2% (19/44), *T. vivax* 13.6% (6/44), *T. brucei* 9.1% (4/44) and two mixed infections: *T. congolense* and *T. vivax* 13.6% (6/44); *T. congolense* and *T. brucei* and *T. vivax* 20.5% (9/44) (Table 1). The prevalence of trypanosomosis varied among sampling zones (Table 1). The highest was recorded in the tsetse-infested valley 35.1% (34/97) and the lowest in the plateau 4.3% (4/92). Highly significant difference was observed between the plateau and the tsetse infested valley ($P < 0.001$), whereas no significant difference was observe between the plateau and the buffer zone ($P = 0.051 > 0.05$). The prevalence in the buffer zone was 5.3% (6/113). The proportion of *T. congolense* infection was higher on the plateau (50%), buffer zone (50%) and infested valley (41.2%), while *T. brucei* was proportionally lower in the three zones, particularly in the tsetse-infested valley (9.1%) (Table 1). *T. vivax* infection was observed on the plateau (25%) and in the tsetse infected valley (14.7%). However, *T. congolense* was significantly higher than *T. vivax* ($P < 0.001$).

3.2 PCV

40.9% (18/44) of positive animals reveled PCV readings less than 25%, whereas 43.2% (19/44) of positive animals showed PCV readings higher than 28%. 19%(49/258) with PCV readings below 25% was negative for trypanosomes. The mean PCV for sampling herds was 29.2±5.8 and the proportion of animals with PCV less than 25% was 22.2% (67/302). The PCV of positive and negative animals ranged between 16-40% and 15-46% respectively. The mean PCV of negatives animals (29.6±5.7%) was significantly higher (P = 0.003 <0.05) than the average PCV of positive animals (26.9±6.4).

3.3 Sex, age, race, colour, and weight

74.8% (226/302) of sampling animals were females, whereas 25.2% (76/302) were males. Prevalence was 12% (27/226) for female, while 22.4% (17/76) for male. Highly significant (P < 0.001) difference was observed between the two sexes. The mean PCV of positive female was (28±6.6%), whereas for positive male it was (25.2±5.8%), no significant difference (P = 0.08 > 0.05) was found to exist between positives animals of the two sexes.

The proportion of sampling animals less than 5 years was 49.7% (150/302), their prevalence was 17.3% (26/150) and the mean PCV of those positive animals was 28.7±5.8%. Moreover, prevalence of animals older than 5 years was 11.8% (18/152) and they're mean PCV 24.3±6.4%. Significant difference (P = 0.01 < 0.05) was found to exist between the mean PCV of positives animals of the two groups.

By race of breed present on the Adamaoua plateau the prevalence was 5.3% (5/95), 20.6% (30/146) and 16.4% (9/55) for Goudali, white and red foulani respectively. Mean PCV for animal was 30.5±5.5%, 28.6±6.2%, 28.7±5.4% and 26.7±5% for Goudali, white foulani, red foulani and Charolais respectively. No significant difference (P = 0.43 > 0.05) was found to exist between the mean PCV of white and red foulani, whereas significant difference (P = 0.326 < 0.05) was found to exist between the mean PCV of Goudali and others breeds present on the plateau. For positive animals, mean PCV was 27.6±7%, 26.1±6.6% and 29.1±5.3% for Goudali, white and red foulani respectively. No significant difference was found to exist between the mean PCV of those positive herds.

Prevalence was 26.3%, 19.4% and 6.4% for animals with black, white and brown skin respectively. Mean PCV for positive animals were 26.5±6.7%, 28±5.8% and 28±6.1% for black, white and brown skin respectively. No significant difference (P = 0.29 > 0.05) was found to exist between the mean PCV of positive white and brown animals, and also between brown and black animals (P = 0.5 > 0.05).

Minimum observed weight was 99 kg and the maximum 554kg. The mean weight was 291.6±76kg. The proportion of positive animals with a weight less than 291 kg was 72.7%(32/44) and their prevalence was 21.2% (32/151), whereas this proportion was 27.3% (12/44) for animals with weight higher or equal than 291 kg and the prevalence was 8% (12/151). Highly significant difference was observed between the two groups (P < 0.001).

4. Discussion

Animals in all the three zones were infected by trypanosomes, most often by *T. congolense.* It was also determined that the trypanosomosis prevalence progressed from

the tsetse infested valley to the plateau. Fifty per cent of infections in the plateau and the buffer zone were due to *T. congolense*, whereas it was 42.1 per cent in the tsetse-infested valley. Several possible reasons could be forwarded to the presence of *T. vivax* (16.7%) infections on the Adamaoua plateau.

The presence of other potential *haematophagus* insect vectors other than tsetse flies; mechanical vector insects such *Stomoxys spp., Tabanus spp.* and *Haematopota spp.* are also abundant in these zones[30], and the presence of game animals such as antelopes in the region. Antelopes are generally accepted to be reservoir hosts of *T. vivax* from which the infections are transmitted to domestic ruminants[33]. According to Kidanemariam *et al.*[34], it is considered that the synergistic effect of the conditions mentioned above contributed for the higher *T. vivax* infection of cattle.

The mean PCV observed was 29.2±5.8 and the proportion of animals with PCV less than 25%, doorstep classically consider as a pathologically sign of illness by Baeur *et al.*[31] was 22.2%. The proportion of animal Anaemia, which is best measured by PCV, remains one of the indicators of trypanosomosis in cattle[35,36]. In our study, however, only 40.9% of parasitologically positive animal animals revealed a mean PCV values less than 25%, whereas 43.2% of positive animals showed PCV values higher than 28%, which is beyond the normal minimum PCV[37]. This finding can be explained by the fact that the microhaematocrit buffy coat technique of detecting trypanosomes in the blood is more sensitive, since it detects slight infections, than that of direct smear examination[38]. Earlier reports on the evaluation of the sensitivity of different diagnostic tests have shown to be in decreasing order, that of microhaematocrit > thick blood smear > thin film > wet film[39].

The mean PCV was 29.2±5.8 and the proportion of animals with PCV less than 25% was 22.2% (67/302). The PCV of positive and negative animals ranged between 16-40% and 15-46% respectively. The mean PCV of negatives animals (29.6±5.7%) was significantly higher than the average PCV of positive animals (26.9±6.4) ($P<0.05$).

The microhaematocrit buffy coat technique has the advantage that it indicates the general condition of the animal by PCV determination. Nevertheless in areas like Adamaoua plateau where other agents causing anaemia prevail[30,40], PCV alone may not be the indicator of choice for detecting trypanosome infections.

On the Adamaoua plateau, the lower PCV reading add to trypanosome negative finding in 22.2% of the animals examined would probably suggests the concomitant occurrence of other anaemia causing factors, presumably tick infestation, *helminthosis, haemoparasitosis*[40] and nutritional deficiencies.

The number of sampling herds in this study was small 0.41% (302/74,559), hence these results have to be interpreted with caution and larger surveys need to be carried out in other divisions of Adamaoua plateau (Vina, Djerem, Mbere and Mayo Banyo) in order to confirm these results.

Was the barrier of bovine's treats with insecticides effective? With prudence we can advance that probably this barrier had a certain degree of effectiveness. Because ten years after the end of massive pulverizations the parasitological prevalence on the plateau is significantly weaker than in the tsetse infested valley. But the trypanosome risk

remains real on the plateau. The fact that the rate of prevalence observes in the plateau with strong predominance of *T. congolense* could reveal the real existence of the *Glossina spp.* in this zone presumably unscathed. One reason could be an infestation during transhumance or a re-infestation of the pastures by the transhumants animals, or also an incomplete eradication of the tsetse during the campaign. As long as tsetse flies are present on the Adamaoua plateau, trypanosomosis remains a continuing problem inhibiting the full utilization of grazing resources in the region; therefore one of the ideal strategies to reduce tsetse pressure could be the association of *"herds traps"* with a well-maintained network of impregnated screens and traps.

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EVALUATION OF PINHOLE CASTRATION AS AN ALTERNATIVE TECHNIQUE FOR GOAT STERILIZATION

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EVALUATION DE LA CASTRATION PINHOLE COMME UNE TECHNIQUE ALTERNATIVE POUR LA STÉRILISATION DES BOUCS

Sommaire

La ligature *in situ* du cordon spermatique (Castration Pinhole) a été décrite comme une technique originale peu invasive de castration des veaux. La présente étude avait pour objectif d'évaluer le stress, la morphologie des testicules et l'efficacité de la méthode sur des boucs adultes cliniquement sains. Les animaux (n = 15, âgés de 1 à 1,5 ans) ont été divisés en 3 groupes de 5. Groupe 1 = contrôle (aucun traitement), groupe 2 = castration chirurgicale et groupe 3 = castration pinhole. Les animaux ont été surveillés quotidiennement à travers des examens cliniques de stress et d'un dosage du cortisol plasmatique. L'efficacité de la méthode a été évaluée par l'étude des caractéristiques séminales et des changements morphologiques de testicules. Les boucs qui ont subi la castration pinhole ont montré une élévation du cortisol plasmatique et des le 7ème jour, ont été azoospermiques et ont manifestés l'atrophie testiculaire. Les sections histologiques ont montré une nécrose ischémique complète.

Nous avons conclu que la castration pinhole est applicable chez les boucs car elle est simple, peu invasive, efficace et moins stressante par rapport à la chirurgie. Elle peut être employée comme une alternative convenable à la castration chirurgicale chez les chèvres.

Mots clés: Castration pinhole, stress, morphologie des testicules, chèvres adultes

Summary

In situ spermatic cord ligation (Pinhole castration) has been described as a novel, minimally invasive technique in calf castration. The current study evaluated stress, testicular morphology and effectiveness of the method in clinically healthy adult goats. Bucks (n = 15, 1-1.5 years old) were divided in to 3 groups of 5. Group 1 = control (no treatment), group 2 = surgical castration and group 3 = pinhole castration. In situ spermatic cord ligation (pinhole technique) was performed in accordance with the procedure described by Ponvijay (2007). Animals were monitored through daily clinical examinations for behavioral indicators of stress in addition to assay of plasma cortisol. Effectiveness of method was assessed through evaluation of seminal characteristics and testicular morphologic changes. All pinhole castrated goats showed elevation in plasma cortisol and by day 7, were azoospermic and manifested testicular atrophy. Histological sections revealed complete ischemic necrosis.

We concluded that pinhole castration is applicable in goats for it is simple, minimally invasive, effective and less stressful compared to surgery. It may be employed as a suitable alternative to surgical castration in goats.

Key words: Pinhole castration, stress, testicular morphology, adult goats

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Introduction

Castration of food animals is a common practice in various livestock production systems. On caprine farms, castration is particularly important in improvement of carcass quality through its role in elimination of buck taint[1]. Castration is also pivotal in controlled breeding for rapid genetic improvement. Conventionally, castration is often accomplished by surgical removal of the testes or rendering them dysfunctional in situ by clamp (Burddizzo) application[2]. Surgery and the clamp methods of castration cause severe pain to the animals and predispose to postoperative complications[3]. Their applications also require skilled or trained personnel and are relatively expensive.

To minimize pain and risks associated with conventional castration methods, other less invasive approaches such as intratesticular injection of irritant chemicals and more recently, *in situ* spermatic cord ligation, have been tried[1,3,4]. *In situ* spermatic cord ligation (Pinhole castration) is reportedly a novel and minimally invasive technique in calves[4]. There is however, no published report about its application for castration in goats of any age. Additionally, stress and systemic responses attributable to spermatic cord ligation has not been assessed. The current study evaluated the stress and effectiveness of pinhole castration in adult goats.

Materials and methods

Animals

Fifteen Mubende goat bucks (1-1.5 years old) were used. Before commencement of experiments, the bucks were certified healthy by a through clinical examination including palpation of the testes and related structures for any apparent abnormalities. The bucks were grazed on natural pasture supplemented with crop residues, leafy branches and maize bran containing mineral supplements with free access to fresh drinking water. The bucks were divided into 3 groups (of 5). One group served as control (no treatment) while the second group was castrated by surgery[2]. The third group was castrated by *in situ* spermatic cord ligation[4].

Monitoring treated goats

Each animal was subjected to daily complete clinical examination to identify behavioral/clinical indices of stress such as abnormalities of posture, demeanor and appetite. Rectal temperatures and scrotal circumferences were also recorded. Blood samples were collected from the jugular vein into heparinised vacutainers 2 weeks before experiments, then on the 2nd, 7th and 14th days after treatments for assay of cortisol. The samples were collected between 8.00 – 9.00 a.m to avoid circadian variations in plasma cortisol. Samples were centrifuged at 1500g for 15 minutes and plasma harvested into serum vials for immediate assays. Quantitative determination of cortisol in plasma was done using commercial competitive ELISA kits (Cortisol Elisa GmbH, Wiesbaden, Germany), according to the manufacturer's instructions.

Semen evaluation

Using a ram electro-ejaculator (Medata Systems Ltd., United Kingdom), semen was collected from each goat a week prior to treatment then 28 days after. Immediately after collection, ejaculate colour, individual

sperm cell motility and concentration were assessed[5].

Gross and microscopic evaluation of testes

After 30 days, the testes were recovered by surgery and their weights, lengths and circumferences measured before subjected to histopathology after fixing in Bouin's solution and 3-5 µm sections stained with Haematoxylin and Eosin (H&E) for examination under light microscope.

Statistical analysis

Kruskal-Wallis analysis of variance (paired t-tests) was performed to compare responses within and between groups. P-values < 0.05 were considered significant.

Results

Clinical parameters

Over the 30-day experimental period, 42% of pinhole-castrated goats showed reduced appetite compared to 48% in the surgery group. Also, 45% of pinhole-castrated goats manifested gait abnormalities as opposed to 50% in the surgery group. There were however, no significant ($p > 0.05$) differences in the rankings of appetite, body condition score and gait/posture between pinhole and surgery groups throughout the experimental period (Table 1). Compared to controls, goats that underwent pinhole castration had significant ($p < 0.05$) rise in mean rectal temperatures (from 38.6 to 39.5°C) by day 2 after treatment. Between days 2 to 7 following pinhole treatment, mean rectal temperature remained high (39.1°C; $p > 0.05$), then significantly ($p < 0.05$) dropped to 38.6°C between days 7 and 14 after treatment. Similarly for surgery, mean rectal temperatures significantly ($p < 0.05$) rose to 39.7°C on the 2nd day after surgery compared to pretreatment values (38.6°C). Unlike pinhole castration, rectal temperatures had significantly ($p < 0.05$) dropped to 38.6°C by the 7th day following surgery (Table 2).

Changes in scrotal circumference

No significant change ($p > 0.05$) in mean scrotal circumference amongst control goats was observed before (21.5 cm) and after (22.3 cm) 30 days form start of experiments (Table 3). Amongst the pinhole-castrated goats, there was significant ($p <0.05$) increase in mean scrotal circumference from 21.3 cm before treatment to 25.3 cm by day 2 after treatment. Mean

Table 1: Changes in appetite and gait following treatments

Treatment	Animals with reduced appetite (%)				Total (%)	Animals with abnormal gait (%)				Total (%)
	D0	D2	D7	D14		D0	D2	D7	D14	
Control	0	0	0	0	0	0	0	0	0	0
Pinhole	0	19	17	6	42*	0	20	19	6	45*
Surgery	0	22	20	6	48*	0	24	20	6	50*
TOTAL (%)	0	41	37	12		0	44	39	12	

**No significant difference (p >0.05) in percentages of animals showing reduced abnormal gait amongst the pinhole or surgery-treated goats.*

Table 2: Changes in rectal temperatures in three treatment groups at different days

Treatment	Days	Mean rectal temperature (°C)	P-Value
Control	Day 0	38.6	
	Day 2	38.6	0.58[a]
	Day 7	38.7	0.17[a]
	Day 14	38.6	0.44[a]
Pin Hole	Day 0	38.6	
	Day 2	39.5	0.017[a]
	Day 7	39.2	0.117[b]
	Day 14	38.6	0.011[c]
Surgery	Day 0	38.7	
	Day 2	39.7	0.002[a]
	Day 7	39.1	0.039[c]
	Day 14	38.6	0.031[c]

There were significant elevations in rectal temperature following surgery or pin-hole castration: a = significant elevation in rectal temperature, b = no significant drop in rectal temperature after treatment, c = significant drop in rectal temperature after treatment.

scrotal circumference subsequently reduced significantly ($p < 0.05$) from 22.3 (day 7) to 19.8 cm (day 14). Scrotal circumferences for surgically castrated goats were not measured.

Changes in plasma cortisol levels

Mean plasma cortisol concentration in control goats was 2.78 ng/ml. As shown in Table 3, there was a drastic ($p < 0.05$) increase in mean plasma cortisol levels by the 2nd day after treatment for both the pinhole (2.89 - 17.0 ng/ml) and surgery (2.80 - 25.1 ng/ml) castrated goats. Between days 2 and 7 after treatments, there was significant ($p < 0.05$) reduction in plasma cortisol levels from 17.0 to 10.4 ng/ml for pinhole-castrated goats and 25.1 - 12.2 ng/ml for surgery-castrated goats. By the 14th day, mean plasma cortisol levels had dropped to 3.1 ng/ml and 3.4 ng/ml for pinhole and surgery-castrated goats, respectively.

Seminal characteristics

Changes in sperm motility and concentration are shown in Table 4. By 14th day after treatments, no sperm presence (motility) was recorded for both pinhole and surgical castration methods, while in the control goats, there was no significant

Table 3: Between group comparison of scrotal circumference, plasma cortisol and sperm count in the three treatment groups at different days.

Variable	Day	Comparison		P-value		
				Pinhole	Surgery	Between group diff
Scrotal circumference	Day 0	Control Pinhole	vs	1.000 -	0.639 0.501	0.307
	Day 2	Control Pinhole	vs	0.000 -	0.000 0.000	0.0000
	Day 7	Control Pinhole	vs	0.189 -	0.000 0.000	0.0000
	Day 14	Control Pinhole	vs	0.012 -	0.000 0.000	0.0000
Plasma cortisol	Day 0	Control Pinhole	vs	0.321 -	1.000 1.000	0.2582
	Day 2	Control Pinhole	vs	0.000 -	0.000 0.001	0.0000
	Day 7	Control Pinhole	vs	0.000 -	0.000 0.035	0.0000
	Day 14	Control Pinhole	vs	0.827 -	0.054 0.410	0.0533
Sperm count	Day 0	Control Pinhole	vs	1.000 -	0.282 0.631	0.2126
	Day 14	Control Pinhole	vs	0.000 -	0.000 1.000	0.0000
	Day 30	Control Pinhole	vs	0.000 -	0.000 1.000	0.0000

difference ($p > 0.05$) in mean percentage motility before (76%) and 30 days later (77%).

Amongst control goats, there was reduction ($p < 0.05$) in sperm concentration from mean of 2.58 to 2.39 x 109 cells/ml on day 14 before slightly increasing ($p < 0.05$) to 2.43 x 109 cells/ml by day 30. In both the surgery and pinhole-castrated goats, there was complete azoospermia ($p = 0.00$; 0 x 109 cells/ml) by 14th to 30th days after treatment.

Gross and histological changes

Grossly, ligated testes were significantly ($p < 0.05$) swollen from 21.3 cm before treatment to 25.3 cm by day 2 after treatment. Swellings subsequently

Table 4: Changes in sperm motility and counts in three groups at different days

Treatment	Days	Motility (%)	P-Value	Counts (x 10^9 cells/ml)	P-Value
	Day 0	76		2.58	
			0.38		0.010
	Day 14	74		2.39	
			0.37		0.300
Control	Day 30	77		2.43	
	Day 0	76		2.60	
			0.00		0.00
	Day 14	0.0*		0.00*	
Pin Hole			1.00		1.00
	Day 30	0.0*		0.00	
	Day 0	77		2.86	
			0.00		0.00
Surgery	Day 14	0.0*		0.00*	
			1.00		1.00
	Day 30	0.0*		0.00*	

** No sperm motility or cells detectable ($p < 0.05$) by day 14 after surgery or pinhole castration.*

Table 5: Changes in mean weight, length and width of testes of treated goats

Treatment	Mean weight ± SD (gm)	Mean length ± SD (cm)	Mean width ± SD (cm)
Control	98.3 ± 1.7	11.4 ± 0.9	18.8 ± 1.3
Pinhole	47.6 ± 0.8*	5.5 ± 1.1*	12.3 ± 1.6*

**Mean testicular weight, length and width were significantly different ($p < 0.05$) from control.*

subsided significantly ($p < 0.05$) from 22.3 cm on day 7 to 19.8 cm by day 14 (Table 3). By day 30, all ligated testes were atrophied and weighed significantly ($P < 0.05$) less than control testes (Table 4). Histological sections of control testes revealed intact seminiferous tubules (Figure 1A). Testes with spermatic cord ligation on the other hand showed degenerative changes typical of ischemic necrosis (Figure 1B).

Discussion

All physical methods of castration (surgery, burdizzo and rubber ring) provoke pain related behavioral responses such as dullness, abnormal gaits, and reduced feed intake[6,7]. In this study, a number of surgery and pinhole-castrated goats showed reduced appetite and abnormalities of gait though the rankings in alterations of appetite,

body condition and gait/posture were not significant between the surgery and pinhole-treated goats. It is therefore apparent from behavioral indicators that pinhole castration provokes pain/stress in goats to magnitudes comparable to surgery. Ponvijay[4] also reported no behavioral differences between intact and pinhole-castrated goats. Behavioral indicators, being subjective measurements are, however, not accurate measures for comparing stress or pain intensities[6].

In the current study, there was elevation in mean rectal temperatures of both surgery and pinhole-castrated goats within 24-48 hours after treatments. Inflammatory afflictions of the scrotum or testes often show symptoms including a febrile state disturbance with the animal generally dull, inappetent and showing a rise in temperature[8]. Total ischemia caused by ligation results in tissue necrosis[9]. In every instance of tissue death, there is an accompanying inflammatory process necessary for resolution of the tissue defect. There was a more drastic decline in mean rectal temperature from peak in the surgery group compared to pinhole-treatment group.

This is suggestive that goats castrated by surgery recover faster than their pinhole castrated counterparts. A similar observation was made in bull calves castrated using lactic acid, where healing time in chemically castrated calves was nearly double that of those castrated by surgery[8]. A clean surgical incision heals faster by primary intention[10]. Chemical and pinhole castration methods on the other hand, cause coagulative necrosis of the testes whose resolution are protracted and are accompanied by fibrosis akin to secondary intention healing[3,4].

Assay of plasma cortisol and by inference pain/stress showed that both surgery and pinhole castration elicited substantial cortisol responses in the goats. Surgery was however responsible for greater cortisol response compared to pinhole castration. This is consistent with the general belief that castration is one of the most stressful procedures in livestock regardless of the method employed though the onset, magnitude and duration of stress or cortisol response may vary with method of castration[6,7,11]. On the basis of cortisol response, pinhole castration was apparently less stressful than surgical castration.

No progressive individual motility of spermatozoa was observed in all the pinhole or surgically-castrated goats from 7 days after treatments. Likewise, complete azoospermia was observed at the same time. A study of effects of vasectomy in bucks also revealed azoospermia [7] days after surgery[5]. A similar observation was also made in vasectomised rams[12]. Like vasectomy, spermatic cord ligation blocks the flow of spermatozoa from the testes/epididymis to the upper duct system in addition to causing ischemic testicular necrosis that leads to complete cessation of spermatogenesis. Acute ischemia through torsion or ligation of spermatic cords for as little as 5 hours, followed by reperfusion, causes irreversible loss of spermatogenesis[9].

In the present study, mean weight, length, width and circumference of ligated testes were significantly reduced compared to that of control goats. In a related study on calves, testes whose spermatic cords were ligated were significantly lower in weights than pair matched controls 30 days after ligation4. Similar findings were observed in pigs and Awassi lambs following chemical castration[13]. Testicular atrophy is characteristic of conditions that lead to degeneration or dysfunction of the testis[3]. Histopathology revealed ischemic coagulative necrosis of the testes. This is consistent with similar findings following laparoscopic

castration of equids[14,15].

On the basis of plasma cortisol, seminal and architectural responses, we conclude that pinhole castration in goats is effective and provoke stress responses comparable to surgery. The method may hence be adopted for wider application as a simpler, less invasive and cheaper alternative to surgical castration.

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THE SEMEN CHARACTERISTICS OF WEST AFRICAN DWARF BUCKS INFECTED WITH *LISTERIA MONOCYTOGENES*

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LES CARACTÉRISTIQUES DU SPERME DES BOUCS NAINS DE L'AFRIQUE OCCIDENTALE (WAD) CONTAMINÉS PAR LA *LISTÉRIA MONOCYTOGENES*

Sommaire

L'effet de la *listeria monocytogenes* sur les paramètres de spermes de huit boucs nains de l'Afrique occidentale a été étudié après une inoculation intra-scrotale de la *listeria monocytogenes* en bouillon de culture. Le volume de sperme collecté (ml) et le taux de motilité (%) des spermes a diminué de façon significative (P<0,05) par rapport à leurs valeurs de pré infection de 0,25 ml et de 84,1% à une valeur post-infection de 0,3 ml et 75,9%.

En outre, le rapport morts-vivants exprimé en pourcentage de longévité s'est également montré significativement plus faible (P <0,05) en post-infection (85,7%) comparée à la pré infection (90,8%). il a été observé (P<0,05) une détérioration statistiquement significative du volume de spermes, de leur motilité, longévité, concentration et de la morphologie.

L'infection par *Listeria monocytogenes* a des effets néfastes sur la viabilité de sperme et les boucs nains mâles contaminés par l'infection ne sont pas appropriés pour la reproduction et l'insémination artificielle.

Summary

We have investigated the effect of *Listeria monocytogenes* on the sperm parameters of eight West African dwarf bucks after intra-scrotally innoculating adequately characterised broth culture of *Listeria monocytogenes*. The volume of semen collected and the percentage motility of the sperm significantly dropped (P<0.05) from their respective pre-infection values of 0.25ml and 84.1% to post-infection value of 0.3ml and 75.9%. Furthermore, live-dead ratio expressed as percentage liveability was also observed to be significantly lower (P<0.05) for the post-infection (85.7%) than for the pre-infection (90.8%).

We observed statistically significant (P<0.05) deterioration in semen volume, sperm motility, liveability, concentration and morphology.

It is therefore concluded that infection with *Listeria monocytogenes* have adverse effect on semen viability and bucks with the infection are not suitable for breeding and artificial insemination procedures.

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Introduction

Listeria monocytogenes is a facultative intracellular pathogen widely found in nature. Although, Listeriosis is known to be primarily foodborne, characterised by septicaemia and the formation of multiple visceral abscesses and meningoecephalitis, elegant studies have demonstrated that the pathogen can cause localised infection in the absence of invasive disease and associated mortality. As a result of its unique characteristic, *Listeria monocytogenes* has been used as a model organism for the study of intracellular parasitism.

Microbial agents have been demonstrated to affect the male reproductive function causing the agglutination of motile sperm[1], reducing ability for the acrosome reaction[2]. Deterioration in spermatogenesis, obstruction of the seminal tract and defect of spermatozoa function has been suggested to be consequences of activation of seminal plasma white blood cells or cellular reaction against microbial agents, as well as direct influence of pathological bacterial strains on gametogenic cells[3].

Pathological bacterial strains are known to directly influence sperm cell structure and function[4], thereby decreasing the fertility of male animals. The level of fertility level of fertility exerts considerable influence on the efficiency of the fertilization procedure, and thus also on reproductive performance

In this paper, we report that testicular inoculation of Listeria monocytogenes can induce deteriorative changes on the spermiogram of West African Dwarf buck.

Materials and Methods

Location of study

This study was carried out at the Veterinary Teaching Hospital, Large Animal Ward II of the Faculty of Veterinary Medicine, University of Ibadan; located at latitude 07^{O}N and latitude 03^{O}50E, with an average humidity of 80%, average ambient temperature of 27^{O}C and a total annual rainfall of 48 inches.

The experimental animals

The study was conducted on eight healthy West African Dwarf Bucks. The weights of the animals were between 6.4-10.0Kg as obtained by means of a suspended graduated weighing scale. They were between 12 - 18 months of age (as estimated by the dentition).[5]

The management of the animals

The bucks were allowed a period of 1 week for acclimatization after arrival. They were housed in concrete, well- ventilated and roofed pens with four animals in each pen. The pens were cleaned daily and the goats were kept intensively (008-1800hr) throughout the period of the experiment (i.e. Zero grazing).

The goats were fed guinea corn offal and Cassava (*Manihot esculata*) peelings and hand – cut elephant grass (*pennisetum purperum*) and centrosema. Fresh water was provided *ad libitum*

The animals on arrival were dewormed using Levadex® injectable preparation of levamisole (Pantex Holland B.V) at a dose rate of 10mg/kg body weight, subcutaneously and Albidol® bolus (containing albendazole) given per os at recommended dosage(Concept

Pharmaceuticals LTD, Mumbai).

The animals were also given prophylactic antibiotic therapy using Terroxy 5%, injectable preparation of Oxytetracycline (SKM Pharma India) at the recommended dosage.

The inoculation of Listeria monocytogenes into the animals

The broth culture of *Listeria monocytogenes* (0.1ml) was inoculated intrascrotally in each buck, care taken not to inject the testes.

The organism had been isolated from some milk samples (from some cows in a farm in Oyo State) and adequately characterized[6]

Semen collection

Semen was collected from the bucks twice before inoculation (once weekly) and thrice, 2 weeks after inoculation (once weekly) using the electro-ejaculation method[7, 8]

Semen examination

Semen samples obtained were promptly analysed by conventional methods[7] for the following: colour, volume, mass activity, motility, percentage liveability, sperm concentration and morphology.

Colour was determined by visual assessment and the volume of the ejaculate measured in a graduated collection tube. Mass activity was determined[9].

Semen smears were made and stained with Eosin – Nigrosin stain for determination of percentage liveability and stained with wells and Awa stain to observe morphology of sperm cells[10].

The sperm concentration was determined by use of the improved Neubaeur haemocytometer [7, 11].

Data analysis

The data generated were analysed with the following tools-test of Homogeneity of variances, multiple comparison and Analysis of Variance. The analysis was done using the SPSS computer software package.

"Pre- infection" refers to before the study animals were infected with *Listeria monocytogenes.*

"Post-infection" refers to after the study animals had been infected with *Listeria monocytogenes.*

Result

Semen parameters in this study indicated that the Volume of semen collected and the percentage motility of the sperm cells significantly dropped ($P<0.05$) from their respective pre-infection values of 0.25ml and 84.1% to post-infection value of 0.31ml and 75.9%. Furthermore, Live-dead ratio expressed as percentage liveability was also observed to be significantly lower ($P<0.05$) for the Post-infection (85.7%) than for the Pre-infection (90.8%).

Sperm cell morphological abnormalities observed during the study include, tailless head, headless tail, rudimentary tail, bent tail, curved mid-piece, curved tail, bent mid-piece, coiled tail.

The abnormalities that were observed to significantly increase ($P<0.05$) from their pre –infection values to higher post-infection values were; Spermatozoa with tailless head (26 to 43), Sperm cells with headless tail (26 to 32), Sperms cells with bent tail (31 to 40), curved mid-piece Sperm cells (39 to 55), bent mid-piece (39 to 57) and sperm cells with coiled tail (0 to 11).

However, no significant difference (P>0.05) were observed in the Pre- infection and the post-infection values of; sperm cells with rudimentary tail (2 to 3) and the sperm cells with curved tail abnormalities (32 to 30).
In all, there was a significant decrease (P<0.05) in the total number of sperm cells from the pre-infection value (3070) to Post infection value (2880).

Meanwhile, the total number of sperm cells with abnormalities significantly increased (P<0.05) after the infection with Listeria monocytogenes (271) than before the infection (195).

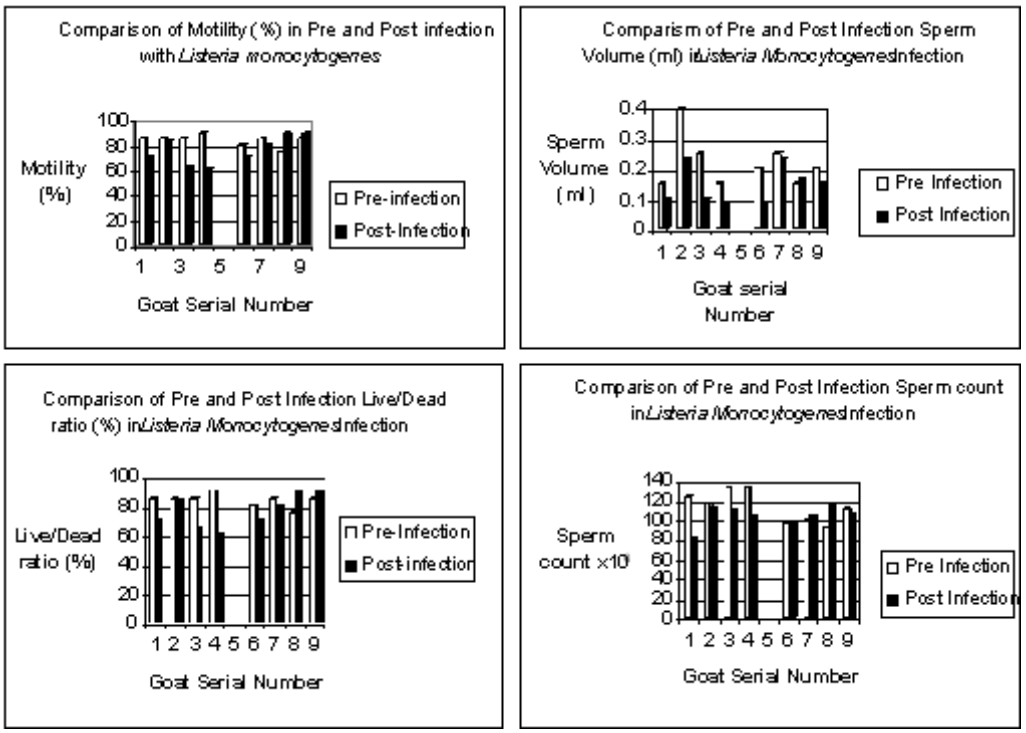
Tables 1 & 2 and figure 1 summarizes the relationship between intra-testicular *Listeria monocytogenes* infection and semen quantity and quality.

Table 1: Seminological analysis of Pre-infection ejaculates from healthy West African Dwarf buck

Goat Number	Mass activity	Motility	Live/Dead Ratio (%)	Colour	Volume (ml)	Sperm count $x10^6$
G1	++	85	93	Creamy	0.15	124
G2	+++	85	90	Milky	0.4	116
G3	++	85	93	Creamy	0.25	133
G4	++	90	95	Creamy	0.15	133
G6	+++	80	86	Milky	0.2	97
G7	++	85	90	Opalescent	0.25	99
G8	++	75	89	Creamy	0.15	93
G9	+++	85	90	Creamy	0.2	110
Mean		83.8	90.8		0.22	113.1
Std. Deviation		4.4	2.8		0.08	16.0
Std. Error		1.6	0.1		0.03	5.7

Table 2: Seminological analysis of Post-infection ejaculates from healthy West African Dwarf buck

Figure 1: Comparison of Pre-infection and Post-infection Semen Parameters



Discussion

Testicular Inoculation of *Listeria monocytogenes* has been shown by a previous study to cause destruction of tubular architecture and massive interstitial inflammation[12]. There is also evidence that *Listeria monocytogenes* could induce pathological changes limited to the testis by autoimmune mechanisms[12]. Although this study could not ascertain the pathological changes and the mechanism involved, the observed vital indexes showed that deteriorating changes occurred in the testis post inoculation of viable *Listeria monocytogenes.*

Marked reduction in percentage progressive motility after infection with *Listeria Monocytogenes* observed in this study could suggest that buck with the organism will have lower fertilizing ability, since motility of spermatozoa at the time of collection has been described to be a measure of fertilizing ability of sperm.

However, the reason for lack of change in mass activity of the sperm cell during the experiment could be due to mass action of the semen sample[13]

The change in the semen colour from creamy-milky to milky after infection suggests a slight change in concentration as observed in previous similar studies[9, 13,14]. Since the colour of ejaculate has been demonstrated to be a function of sperm concentration[15], therefore infection with *L. monocytogenes* reduces sperm concentration in the west African dwarf buck.

Furthermore, this study also showed a drop in the semen volume post-infection. This observation could be ascribed to the deleterious effect of the organism on spermatogenesis.

The Drop in percentage liveability after infection may be indirectly be attributed to stress induced by the infection; as observed in similar stressed inducing conditions like vasectomy[13], scrotal insulation[16], castration[17]and starvation[18].

Post infection decrease in percentage liveability (Live-dead ratio) observed during the study suggests decrease in semen fertility of infected buck; since percentage liveability had been shown by earlier studies to be a function of semen fertility[19].

These finding corroborates the result of a study that showed that bacterial infection induces germ cell apoptosis and as such blocks sperm formation[20].

Sperm cell morphological abnormalities that were observed to significantly increase after infecting the buck with *Listeria monocytogenes* could possibly be ascribed to the adverse effect of the organism all stages of maturation of the germinal epithelium from spermatogonia to spermatozoa, as demonstrated in guinea pigs[12].

Although the increase in the formation of abnormal sperm cells were significant in this study, the increase was not as pronounced as those observed under stress conditions like scrotal Insulation[16] and the increase is still within the normal limit of 10% demonstrated in a previous study[7].It is however, difficult to compare this experiment with other studies, like scrotal insulation, orchidectomy and starvation, since the observed differences may be connected to the differences in feeding, management practices, method of semen collection and other inherent individual variability existing within the experimental animals.

This study showed that *Listeria monocytogenes* infection has adverse effect on the total number of sperm cells produced as evident from the fall in pre-infection value of sperm cells count. The decrease in sperm cells quantity and quality suggests disruption of normal spermatogenesis and germ cell apoptosis, possibly due to massive interstitial inflammation and testicular degeneration induced by the organism. Invasion of micro-organisms into the male genital tract has also been established to induce oxidative stress and attract pro-inflammatory cytokines with the all beneficial and pathological consequences.[21,22,4,23],

Conclusion

This three weeks study on the effect of *Listeria monocytogenes* on the spermiogram of West African Dwarf buck reveals reduction in the motility of spermatozoa, Live-dead ratio, quantity and concentration of ejaculate, along with increase in the number of sperm cell morphological abnormalities. These vital changes strongly suggest that infection with this organism have adverse effect on semen viability.

This study strongly suggests that buck with listeriosis are not suitable as breeding animal and should not be selected for use in artificial insemination.

Furthermore, this preliminary investigation serves as a background for subsequent studies on the effect of infection on fertility parameters of West African Dwarf bucks.

However, further studies are required to ascertain the full extent and mechanism of the effect of listeriosis on male reproductive

performance, especially if they are conducted under prolonged condition.

In addition, a good comparison of spermiogram under experimental infection and natural *Listeria monocytogenes* infection will be of great value.

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CARACTERISTIQUES FERMENTAIRES, INGESTION VOLONTAIRE ET DIGESTIBILITE DE L'ENSILAGE DE *PENNISETUM KING GRASS* CHEZ LE MOUTON DJALLONKE

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VOLUNTARY INTAKE AND DIGESTIBILITY OF THE GIANT NAPIER *(PENNISETUM KING GRASS)* SILAGE FED TO DJALLONKE SHEEP

Summary

A study was carried out on fermentable properties, voluntary intake and digestibility of *Pennisetum King Grass* silage fed to djallonke sheep. The silage had a pH of 3.8 and the main fermentation end products was 2.8% of total-N for the ammonia nitrogen ($N\text{-}NH_3$), 19.6 g/Kg DM of acetic acid and 22.0 g/Kg DM of ethanol. Dried matter intake was 60.42g $DM/Kg^{0.75}$ per day and digestibility showed no significant differences for dried matter (DM), organic matter (OM), crude fiber (CF) and ether extract (EE). Digestibility was significantly lower ($P<0.01$) for ash. Finally, an average digestibility without significant difference was registered for crude protein (CP). The quality associated with the nutritive value, the energy value for milk production the energy value for meat production, and nitrogen value, confer to the PKG silage qualities of a feed which is able to feed suitably sheep, in time of fodder scarcity.

Keywords: Silage - *Pennisetum* - fermentable characteristics – intake - digestibility – Sheep

Sommaire

Les caractéristiques fermentaires, l'ingestion volontaire et la digestibilité d'un ensilage de *Pennisetum King Grass* (PKG) ont été étudiées chez le mouton djallonké. Cet ensilage était caractérisé par un pH de 3,8 et des teneurs de 2,8% de N-total pour l'azote ammoniacal ($N\text{-}NH_3$) ; 19,6 g/Kg MS d'acide acétique et 22, 0 g/Kg MS d'éthanol. La matière sèche volontairement ingérée a été en moyenne de 60,42 g MS/Kg P0,75/jour. La digestibilité des éléments nutritifs a marqué par rapport à celles du fourrage vert

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correspondant, des différences non significatives pour la matière sèche (MS), la matière organique (MO), la cellulose brute (CB) et les matières grasses (MG). La baisse a été très significative (P<0,01) pour les matières minérales (MM). Enfin, une digestibilité moyenne sans différence significative a été observée pour les matières azotées totales (MAT). La qualité de conservation associée à la valeur nutritive et aux valeurs énergétique et azotée d'une part, et d'autre part, aux valeurs énergétique et azotée, confère à cet ensilage de PKG, les qualités d'un aliment capable, de nourrir les ovins en période de pénurie fourragère.

Mots clés: *Ensilage-Pennisetum*-caractéristiques fermentaires - Ingestion- digestibilité-ovin.

1. Introduction

Les pâturages naturels occupent sous les tropiques, une place prépondérante dans l'alimentation des ruminants domestiques. Leur variation quantitative et qualitative dans l'espace et dans le temps est en rapport avec les conditions climatiques et le dynamisme de la végétation[9]. Elle pose le problème de la disponibilité des ressources fourragères. L'absence de report fourrager accentue en outre les difficultés d'affourragement, particulièrement en période de pénurie fourragère au cours de laquelle, les animaux accusent d'importantes pertes de poids.

La solution à ce double handicap passe par une utilisation rationnelle et intensive des ressources disponibles au niveau local, pour assurer un développement plus rapide de la production de viande et de lait. La maîtrise de l'exploitation et de la conservation des fourrages à haut potentiel de production permettrait d'améliorer de façon substantielle la production des ovins, des caprins et des bovins.

Le *Pennisetum King Grass* (PKG), hybride obtenu à partir du *Pennisetum purpureum* et du *Pennisetum typhoïdes*, introduit au Congo dans les années 1980, pourrait répondre à ces deux exigences. Le potentiel fourrager de cette plante a déjà fait l'objet d'études[8, 12]. Cependant, dans les conditions géo-climatiques de ce pays, la plupart des essais de conservation de fourrage n'ont été réalisés qu'avec les ensilages[3, 4, 10]. En outre, ceux – ci ne se sont limités qu'à l'étude de leur composition chimique sans aborder les aspects relatifs à leur valorisation par les animaux domestiques.

Le présent travail a été entrepris pour combler cette lacune. Il s'inscrit dans le prolongement des recherches antérieures[10] et vise l'étude des caractéristiques fermentaires de l'ensilage de PKG, son ingestion volontaire et partant, son utilisation comme aliment de base chez le mouton djallonké.

2. Matériel et méthodes

2.1 Matériel

2.1.1 Préparation de l'ensilage

L'ensilage a été réalisé avec du fourrage vert, le *Pennisetum King Grass* (PKG) à 19,5% de matière sèche (MS). Ce fourrage obtenu à partir des boutures de 3 à 5 nœuds mises en terre, a été récolté à 105 jours et

haché en particules de 1 à 3 cm de long, à l'aide d'un broyeur à poste fixe.

L'essai d'affourragement s'est déroulé au Laboratoire de Zootechnie du Centre de Recherches Vétérinaires et Zootechniques (CRVZ) à Brazzaville, dans le site décrit dans des essais précédents[11, 10]. Le matériel végétal ainsi préparé a été ensilé sans conservateur pendant une durée de 47 jours, dans un silo - couloir à surface bétonnée ayant une pente de 2 à 3% et préalablement paré d'un film plastique de couleur noire de 2,50 µ d'épaisseur. Le silo avait un volume total de 41.500 litres et répondait aux caractéristiques suivantes :

- Longueur 7,40 m
- Largeur 3,30 m
- Hauteur 1,70 m

La récolte du fourrage et la mise au silo se sont déroulées en une matinée. Le fourrage finement haché a été bien réparti dans le silo, puis tassé par des passages successifs du tracteur. A la fin de l'opération, le silo a été recouvert et fermé au moyen du même film plastique sur lequel des objets lourds ont été déposés.

2.1.2 Les animaux

Quatre béliers Djallonké entiers, provenant du troupeau expérimental, ont été utilisés pour les essais d'ingestion et de digestibilité. Ces animaux d'un poids moyen de 18 Kg constituaient un lot homogène du point de vue du poids. Ils ont reçu préalablement aux essais, un traitement anthelminthique à base de Thiabendazole et ont été ensuite placés dans des cages à bilan individuelles, en stabulation entravée. Le poids initial et final de chaque animal a été mesuré avec une bascule pèse - bétail Marechalle Type PM 300 de portée maximale 300 Kg et de précision moyenne (0,200 Kg).

2.1.3 Régime alimentaire

Deux régimes alimentaires ont été testés pendant l'essai qui s'est déroulé en deux phases, sur une période de 31 jours. La première phase a consisté en un essai d'affourragement en vert au cours de laquelle du *Pennisetum King Grass* (PKG) vert haché en particules de 1 à 3 cm a été distribué. Cette phase a duré 14 jours dont 7 jours d'accoutumance à la ration et 7 jours pour les mesures. L'essai d'affourragement à base d'ensilage de la deuxième phase a duré 17 jours. La période d'accoutumance a pris 10 jours, les animaux ayant mis plus de temps à s'adapter à ce régime, et celle de l'essai proprement dit 7 jours. Dans les deux phases, les périodes d'accoutumance ont permis de fixer la ration journalière à 4 kg de fourrage (vert ou ensilé) distribué à raison de 2 kg le matin à 8H00 et l'après – midi à 16H00. A la fin de chaque période de mesure, le cumul des échantillons prélevés et conservés quotidiennement pour chaque animal et chaque catégorie ((distribué, refus) a permis de constituer un échantillon représentatif sur lequel a porté l'analyse fourragère. Les échantillons de fèces ont été arrosés d'H_2SO_4 à 5% pour éviter les pertes azotées.

2.2 Méthodes d'analyse

Les méthodes officielles d'analyse fourragère de la CEE ont été utilisées pour la détermination de la composition chimique des échantillons de fourrages vert et ensilé. Elles ont porté sur la matière sèche (MS) obtenue par dessiccation à l'étuve à 105°C jusqu'à poids constant, les matières minérales (MM) par incinération à 500°C pendant 5 à 6 heures, les matières azotées totales (MAT) par la méthode de Kjeldahl,

l'Extrait Ethéré (EE) par extraction à l'éther diéthylique, la cellulose brute (CB) par la méthode dite de la station agronomique de WEENDE, du résidu obtenu après deux hydrolyses successives la première en milieu acide et la seconde en milieu alcalin et la matière organique (MO), obtenue par différence entre la matière sèche (MS) et les matières minérales (MM).

La qualité de conservation de l'ensilage a été appréciée à partir du jus d'ensilage obtenu par macération de 100g d'ensilage dans 1000 ml d'eau distillée, puis filtration, après conservation pendant 24 heures au réfrigérateur. Les paramètres suivants ont été déterminés:

- Le pH, mesuré à l'aide d'un pH-mètre à électrodes combinés et à lecture directe
- L'azote ammoniacal (N-NH3) par la méthode de CONWAY
- Les acides gras volatils (AGV) par chromatographie en phase gazeuse (CPG) à partir du jus d'ensilage obtenu selon le procédé indiqué ci-dessus. Les teneurs en acides acétique, butyrique, iso butyrique et propionique ainsi que l'éthanol ont été déterminées. Le chromatographe utilisé était un DELSI, série 30, avec une colonne de séparation en acier inoxydable.

2.3 Méthode de détermination de l'ingestion et de la digestibilité

Le niveau d'ingestion a été déterminé par la quantité de matière sèche volontairement ingérée (MSVI) par jour, exprimé en g de MS par kg de $P^{0,75}$ à partir de la relation (a)

$$I = Qd - Qr \quad (a)$$

Avec I = Quantité de matière sèche ingérée

Qd = Quantité de matière sèche distribuée

Qr = Quantité de matière sèche refusée

Le Coefficient d'utilisation digestive apparent (CUDa) ou digestibilité de chaque substance nutritive a été déterminée par la relation (b)

$$CUDa = \frac{\text{Elément Ingéré} - \text{Elément Fécal}}{\text{Elément Ingéré}} \times 100 \quad (b)$$

Le terme *''élément''* s'applique ici indistinctement à tout élément nutritif considéré.

2.4 Mode de calcul de la valeur énergétique

L'expression de la valeur énergétique en unités fourragères ''lait'' (UFL) et unités fourragères ''viande'' (UFV) a été utilisée. Son mode de calcul est celui proposé par les Nutritionnistes de l'Institut National de la Recherche Agronomique (INRA) de France (7).

La valeur énergétique en unité fourragère (UF) a été calculée selon la formule;

$$UF/Kg\ MS = \frac{2,36\ MOD - 1,20\ MOND}{1.650}$$

Avec:

MOD : Matière organique digestible (en g/kg MS)

MOND : Matière organique non digestible (en g/Kg MS)

La quantité de matière organique non digestible (MOND) est obtenue par la différence entre la quantité de matières organiques totales (MO) et la quantité de matières organiques digestibles (MOD).

Pour tenir compte des différences de rendement et de l'énergie métabolique (EM)

des fourrages pour l'entretien, la lactation et l'engraissement que la formule ci-dessus ne prend pas en compte et sous estime, le système suivant a été utilisé :

- Unité Fourragère ''Lait'' (UFL) qui prend en considération les femelles en lactation, les animaux à l'entretien ou à croissance modérée (< 750 g/jour) ;
- Unité Fourragère ''Viande'' (UFV) appliquée aux animaux à l'engrais avec une croissance supérieure à 750 g/jour.

2.5 Analyse statistique

Le test '' t'' de STUDENT - FISHER a été utilisé pour la comparaison des moyennes. Chaque moyenne a été associée à son écart - type.

3. Résultats

3.1 Composition chimique et caractéristiques fermentaires de l'ensilage de PKG

L'ensilage de *Pennisetum King Grass* (PKG) obtenu a présenté un taux de matière sèche (MS) significativement supérieur à celui du fourrage vert correspondant (Tableau 1). Les taux de matière organique (MO) et de cellulose brute (CB) ont également augmenté de façon significative par rapport aux taux correspondants dans le fourrage vert, avec respectivement des différences de 30 points et 7 points.

Les matières minérales (MM), matières azotées totales (MAT) et l'extrait éthéré (EE) ont par contre accusé une baisse non significative. Les différences étaient respectivement de – 0,4; - 0,44 et – 0,07 points par rapport aux taux correspondants dans le fourrage vert témoin (Tableau 1).

La conservation du PKG après une durée de 47 jours, a donné un ensilage de couleur vert olive à forte odeur aromatique.

Tableau 1 : Composition chimique, valeur alimentaire et ingestion du *Pennisetum King Grass (PKG) vert et ensilé*

NATURE DU FOURRAGE	COMPOSITION CHIMIQUE (en p. 100)						VALEUR ALIMENTAIRE ET INGESTION					
	MS	MM	MO	CB	MAT	EE	UF/ Kg MS	UFL/ Kg MS	UFV/ Kg MS	MAD MS g/Kg		QI g MS/Kg P^{0,75}
(a) PKG vert	17,00±0,04* (c)	6,06±0,01	64,36±0,06 **	32,75±0,15 *	9,62±0,00	3,25±0,13	0,36	0,83	0,60	50,83±6,25		57,25±1,32
(b) PKG ensilé	19,30±0,45 *	5,66±0,01	94,34±0,36 **	39,40±0,04 *	9,18±0,43	3,18±0,18	0,36	0,85	0,62	47,55±1,68		60,42±2,24
Ecart = (b) – (a)	+ 2,3	- 0,4	+ 29,99	+ 6,65	- 0,44	- 0,07	0,00	- 0,02	- 0,02	- 3,28		- 3,17

(c): Matière Sèche (MS) corrigée avec 0,8 point, la MS non corrigée étant égale à 18,5 p. 100

* P < 0,05 Différence significative dans la même colonne
** P < 0,01 Différence très significative dans la même colonne
Matières Minérales (MM), Matière Organique (MO), Cellulose Brute (CB), Matières Azotées Totales (MAT), Extrait Ethéré (EE).
Unité Fourragère (UF), Unité Fourragère Lait (UFL), Unité Fourragère Viande (UFV), Matières Azotées Digestibles (MAD), Quantité ingérée (QI).

Tableau 2 : Caractéristiques fermentaires de l'ensilage de *Pennisetum var king grass*

pH	N-NH3	Acide acétique (g/kg MS)	Acide butyrique (g/kg MS)	Acide isobutyrique (g/kg MS)	Acide propionique (g/kg MS)	Ethanol (g/kg MS)
3,8	2,8	19,6	0	0	0	22,00

Le produit n'a présenté aucune trace de moisissures, ni de dégradation quelconque. La mesure du pH et le dosage des principaux produits terminaux des fermentations, ont donné des résultats qui indiquent un ensilage ayant une qualité de conservation excellente[6, 7] (Tableau 2).

3.2 Ingestion volontaire, digestibilité de l'ensilage de PKG et réponse pondérale des moutons Djallonké

La quantité de matière sèche volontairement ingérée (MSVI) était supérieure à celle du fourrage vert avec 60,42 g de MS/kg $P^{0,75}$ pour l'ensilage de *Pennisetum King Grass* contre 57,25 g de MS/ kg $P^{0,75}$ pour son fourrage vert (Tableau 1). La digestibilité des éléments nutritifs de l'ensilage a présenté quant à elle, par rapport au fourrage vert, des différences non significatives en ce qui concerne la matière sèche (MS), la matière organique (MO) et les matières cellulosiques (Tableau 3). Par contre, la digestibilité des matières minérales a accusé une baisse significative entre le fourrage vert et l'ensilage, tandis que celle des matières azotées n'a pas changé de manière significative. Le gain de poids enregistré par les animaux nourris à l'ensilage de *Pennisetum King Grass* a été de 485 g en moyenne. La réponse pondérale des animaux ayant consommé l'ensilage de PKG a été positive (Tableau 3).

Enfin, la valeur de l'énergie métabolisable a été évaluée à 2,51 Mcal/kg MS pour l'ensilage de PKG contre 2,44 Mcal/kg MS pour le fourrage vert correspondant. Une différence non significative a été observée pour les valeurs énergétiques, notamment les énergies nettes pour la lactation (0,85 UFL/kg MS) et pour la production de viande (0,62 UFV/ kg MS) de l'ensilage de PKG par rapport à celles du fourrage vert correspondant, avec 0,83 UFL/kg MS et 0,60 UFV/kg MS. Par contre, la valeur azotée de l'ensilage s'est avérée légèrement inférieure à celle du fourrage vert témoin avec 47,55 g MAD/kg MS contre 50,83 g MAD/kg MS.

4. Discussion

4.1 Composition chimique et caractéristiques fermentaires

Les légères modifications constatées dans les constituants chimiques de notre ensilage permettent de dire que la composition chimique de l'ensilage de *Pennisetum King Grass* (PKG) a été bien conservée. En effet, la teneur en matière sèche (MS) élevée dans notre ensilage par rapport à son fourrage vert correspondant est en accord avec l'observation selon laquelle, la teneur en MS de l'ensilage est supérieur à celle de la plante verte quand celle – ci est ensilée à un taux de MS inférieur à 20%, à cause des pertes d'eau dans les jus d'écoulement de l'ensilage[5]. Titterton *et al.*[15] font également remarquer que les matériaux ensilés avec moins de 30% de MS créent un environnement totalement anaérobie qui par la suite aboutit

à la perte d'eau et d'éléments solubles qui s'accumulent au fond du silo sous forme d'effluents d'ensilage. La variation rapportée ci – dessus de 2,3 points, pour un fourrage de *Pennisetum King Grass* (PKG) ensilé à 17% de MS, peut même selon ces auteurs, atteindre 4 points, particulièrement dans le cas des fourrages ensilés à 13 – 14% de MS. Dans tous les cas, un taux correct de MS dans le fourrage avant l'ensilage, est un facteur important pour la réussite du processus de fermentation[1].

Concernant les autres constituants chimiques, il a été signalé par ailleurs que le taux de matières cellulosiques de l'ensilage est en général de 5 à 10% plus élevé que celui du fourrage vert correspondant. Cependant, pour des fourrages très humides, les taux de matières azotées et de matières minérales de l'ensilage sont légèrement inférieures à ceux des fourrages verts à cause des pertes signalées plus haut. Les variations des taux de matière organique, de matières azotées et de matières minérales, observées dans notre essai entre l'ensilage de Pennisetum king grass et son fourrage vert corroborent les conclusions de ces auteurs.

En rapport avec les caractéristiques fermentaires, la considération de tout ou partie des critères définis par Demarquilly *et al*.[6], pour apprécier la qualité de conservation de l'ensilage, permet de conclure à un ensilage de PKG de qualité excellente. Les caractéristiques fermentaires avec notamment un pH inférieur à 4,4 sont ceux d'un ensilage présentant une excellente qualité de conservation. Cet ensilage est de loin le meilleur qui ait été réalisé dans les conditions de la zone humide du Congo. Il faut en effet souligner que des ensilages ont été réalisés dans les conditions du pays. Mais, ils avaient tous

Tableau 3 : Digestibilité de l'ensilage de *Pennisetum King Grass* (PKG) comparée à celle du fourrage vert (témoin) et réponse pondérale

Nature du fourrage	Digestibilité de la M.S. (en p. 100)	Digestibilité des constituants chimiques (en p. 100 de MS)				M.O.D	Eléments digestibles (en g MS)		Croissance pondérale du mouton djallonké	
	M.O.	M.M.	M.A.T.	C.B.	E.E.		C.B.D.	M.A.D.	Poids initial (en kg)	Poids final (en kg)
PKG vert (témoin)	67,27±5,62	68,54±5,34	51,39±6,50**	52,84±5,50	79,55±3,47	65,53±6,00	327,28±1,83	160,63±5,03*	50,83±6,25	18,15±1,05	18,82±0,92
Ensilage de PKG	63,89±3,42	66,00±3,24	31,10±2,43**	51,81±2,28	77,17±2,28	68,38±0,52	329,34±15,71	158,01±10,66*	47,55±1,68	17,63±0,83	18,19±0,80

* P < 0,05 Différence significative dans la même colonne
** P < 0,01 Différence très significative sur la même colonne

Matière Organique Digestible (MOD), Celle base Energie Digestible (CBD)

l'inconvénient d'avoir des pH élevés et présentaient des traces d'acides organiques néoformés[3, 4, 2, 10]. Signalons qu'un pH comparable de 4,4 a été obtenu en milieu tropical avec un ensilage d'herbe à éléphant[15], qui est un *Pennisetum* comme le PKG.

L'ensilage de PKG réalisé sans additif dans le même environnement, avait un pH de 4,5 [2]. Il était comme dans notre essai, caractérisé par une absence d'acide butyrique et d'acide propionique. Les acides organiques néoformés toxiques étaient totalement absents dans l'ensilage de PKG de notre essai, traduisant ainsi de bonnes caractéristiques fermentaires et partant, une excellente qualité de conservation.

4.2 Ingestion volontaire, digestibilité de l'ensilage et réponse pondérale des moutons Djallonké

L'amélioration de l'ingestion obtenue avec l'ensilage de *Pennisetum King Grass* (PKG) dans notre essai confirme les résultats obtenus dans le même environnement, qui rapportent un niveau d'ingestion satisfaisant de l'ensilage de PKG chez le mouton[2].

Demarquilly et Dulphy[5] ont signalé l'augmentation de quantités d'ensilage ingérées consécutive à une utilisation correcte de films plastiques de bonne qualité, d'addition de conservateur efficace ou d'un hachage fin. La légère augmentation de l'ingestion (5% en moyenne) observée comme chez le maïs plante entière qui est une plante à bonne ensilabilité, pourrait s'expliquer par le faible degré des fermentations conduisant à la production d'AGV et à la dégradation des protéines. Cette hypothèse est corroborée par l'excellente qualité de conservation observée pour notre ensilage de PKG.

Il convient de signaler que l'ingestion de l'ensilage de PKG de notre essai était largement supérieure à celle observée dans le même environnement. Cependant, la différence observée pourrait être liée à la meilleure qualité de conservation de notre ensilage et au système de double distribution des rations quotidiennes adoptée dans notre essai. La différence de digestibilité, significative, observée pour les matières minérales (MM) peut s'expliquer par les pertes importantes d'éléments minéraux digestibles dans les jus d'écoulement évoqués plus haut. Quel que soit l'élément considéré, matière sèche (MS), cellulose brute (CB), matières azotées totales (MAT) ou extrait éthéré (EE), les valeurs de digestibilité restent supérieures à celles obtenues avec un ensilage de *Pennisetum King Grass* (PKG) dans les mêmes conditions d'environnement, notamment celles de la zone humide du Congo[2]. Cependant, les écarts observés sont généralement faibles et nos résultats sont globalement dans la limite de ceux couramment rencontrés.

Les résultats obtenus concernant la valeur énergétique et la digestibilité de l'ensilage de *Pennisetum King Grass* étaient supérieurs à ceux du fourrage vert correspondant. En outre, cet ensilage distribué aux ovins était aussi globalement bien appété et la réponse pondérale de ces animaux consommant cet aliment était positive. Il peut donc constituer une ration de base pour ces animaux. Ainsi, la technique d'ensilage pourrait aider à la résolution du problème de l'alimentation des ruminants domestiques, particulièrement des ovins, en période de déficit fourrager de saison sèche. Cependant, des études complémentaires s'avèrent encore nécessaires pour bien cerner,

particulièrement, les aspects économiques de l'utilisation d'une telle technique. Les variations non significatives d'une part, des valeurs énergétiques de l'ensilage obtenu par rapport au fourrage vert et d'autre part, la légère baisse de la valeur azotée, qui pourraient être liées aux faibles pertes d'éléments nutritifs dans les jus d'écoulement, laissent suggérer que les qualités du fourrage vert ont été conservés.

Notons que des niveaux énergétiques presque comparables, ont été rapportés pour l'ensilage de *Pennisetum King Grass* et son fourrage vert[2]. Ainsi, la digestibilité et l'ingestion volontaire associées aux valeurs énergétique et azotée, confèrent à l'ensilage de PKG, une bonne valeur alimentaire. La technique d'ensilage conserve bien la qualité du fourrage ainsi que sa valeur alimentaire et l'améliore même à la limite de façon substantielle.

5. Conclusion

La nécessité de trouver une solution au problème d'affourragement des ruminants domestiques, particulièrement en période de pénurie fourragère, appelle l'utilisation de méthodes intensives d'alimentation. L'essai a montré que le *Pennisetum King Grass,* graminée à haut potentiel de production est un fourrage qui se prête bien à la technique de conservation par voie humide. Le produit obtenu présente, en plus d'une bonne composition chimique, associée à un niveau d'ingestion volontaire et une digestibilité acceptables, les caractéristiques fermentaires d'un ensilage susceptible d'être valorisé sans problème, par le mouton Djallonké. La possibilité de produire l'ensilage avec un fourrage tropical et de l'utiliser comme aliment de base des ruminants domestiques dans le contexte du pays a ainsi été démontrée.

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AMMONIATION OF RICE STRAW USING POULTRY LITTER: EFFECT ON NUTRIENT COMPOSITION AND PATHOGENIC MICROBIAL FLORA

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AMMONIATION DE PAILLE DE RIZ UTILISANT LITIERE DE VOLAILLE: EFFET SUR LA VALEUR NUTRITIVE ET LA FLORE MICROBIENNE PATHOGENES

Sommaire

La valeur nutritive et la flore microbienne pathogène de la paille de riz traitée à la litière de volaille dans un essai factoriel 5x3x3 a été évaluée à l'Université Abubakar Tafawa Balewa, à Bauchi, au Nigeria. La paille de riz a été mélangée avec la litière de volaille (RSPL) dans cinq proportions différentes 5:5, 6:4, 7:3, 8:2 et 9:1. L'eau a été mélangée avec chacun des RSPL (1-5) dans trois proportions différentes, 50:50, 60:40 et 70:30, respectivement. Les mélanges ont été conservés dans des fûts métalliques étanche pendant 7, 14 et 21 jours.. La masse microbienne par gramme (UFC/g) était significativement (P <0,05) influencée par la proportion de la paille de riz à la litière de volaille (RS: PL). La plus haute (2,90 x 106) et la plus faible (6.0 x 102) CFU/g ont été enregistrées pour 5:1 et 9:1 respectivement. La période de traitement a influencé de façon significative (P <0,05) les protéines brutes (PB), la cellulose brute (CF), l'extrait éthéré (EE) et UFC/g. Le CP a augmenté alors que les CF et UFC/g ont diminué en fonction des jours de traitement. On peut déduire que le traitement de la paille de riz avec la litière de volaille peut améliorer la valeur nutritive et en même temps réduire les micro-organismes pathogènes dans la paille traitée.

Mots-clés: litière de volaille, paille de riz, agents pathogènes, ammoniation.

Summary

The nutritive value and pathogenic microbial flora of poultry litter treated rice straw in a 5x3x3 factorial trial was conducted at the Abubakar Tafawa Balewa University, Bauchi, Nigeria. Rice straw was mixed with poultry litter (RSPL) in five different ratios; 5:5, 6:4, 7:3, 8:2 and 9:1. Water was mixed with each of the RSPL (1-5) in three different proportions; 50:50, 60:40 and 70:30 respectively. The mixtures were kept in air-tight metal drums for treatment periods of 7, 14 and 21 days respectively. The colony forming unit per gram (CFU/g) was significantly (P<0.05) influenced by ratio of rice straw to poultry litter (RS: PL). The highest (2.90 x 106) and lowest (6.0 x 102) CFU/g was recorded for 5:1 and 9:1 respectively. Treatment period significantly (P<0.05) influenced the crude protein (CP), crude fibre (CF), ether extract (EE) and CFU/g. The CP increased while the CF and CFU/g decreased with days of treatment. It may be concluded that treating rice straw with poultry litter can improve the nutritive value while at the same time reduce the pathogenic microorganisms of the treated straw. An equal proportion of crop residue to poultry litter with an equal amount of water is required to enhance treatment. Treatment period of 21 days is required for effective treatment making the material safe for feeding to livestock.

Keywords: Poultry litter, Rice straw, Pathogens, ammoniation.

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Introduction

Ruminant animals in most African countries are reared on poor quality herbage that can be harvested or grazed from uncultivated land, forest areas, water ways and stubbles left over from the cultivation of crops, particularly cereal straws. Because of the scarce availability of fodder, crop residues and other by-products has become the mainstay for the nutrition of these animals.

The conversion of the biomass of crop residues to animal products is associated with a number of difficulties which have often limited their use. These include, very fibrous nature of crop residues, such that the gross energy they contain is often unavailable to ruminant animals[1]. The low digestibility due to the fibrous nature of the crop residues is often compounded by the high contents of lignin and silica which inhibit rumen microbial fermentation and hence digestion of the material. Nutrients such as nitrogen, sulphur and other minerals essential for both the rumen microbes for fibre breakdown and the host animal are low in crop residues. The consequences for ruminants are low dry matter (DM) intake (1.2kgDM/100kg live weight), low digestibility (30-45%) and low performance[2, 3].

The main treatments for improving the voluntary intake and nutritive value of crop residues and by-products are physical, chemical and biological, of which one or more can be applied[4,5].

Several chemicals have been used to upgrade crop residues[6, 7, 8] and, most of these treatments are not practicable in resource poor countries because of the unavailability of the chemicals due to high cost, scarcity and poor storage facilities.

The use of urea as a source of ammonia to treat crop residues in the tropics has been reported[9, 10, 11] to be very favourable due to the high temperature often experienced in this area, which is a requirement for the hydrolysis of urea to ammonia. 8 had shown that response to treatment depends on urea concentration, period of incubation and environmental temperature.

The use of poultry litter as a precursor of ammonia in treating crop residues has been reported[12, 13, 14]. The treated material unlike supplementation has the combined effect of breaking the lignin bond and adding nitrogen to the crop residue. This study was therefore aimed at investigating the effect of treating rice straw with poultry litter (broiler litter) on the nutrient composition and pathogenic microbial flora of the treated straw.

Materials and methods

Collection of poultry litter and rice straw

The poultry litter used in this study was collected from the Poultry Unit of the Abubakar Tafawa Balewa University (ATBU) research and teaching farm, Bauchi. The litter was removed from broiler pens after the birds had been housed for eight weeks. The litter was milled at the ATBU feed mill to ensure complete mixing of the spilled feed, feathers, excreta and bedding materials.

Rice straw was gathered from already harvested rice farms within Bauchi environment. The rice straw, comprising of the leaf and straw portions, was chopped manually using matchet to a length of approximately 3-5cm.

Treatment of rice straw

The chopped rice straw was mixed with poultry litter in five different ratios; 5:5, 6:4,

7:3, 8:2 and 9:1 and were designated as RSPL 1, RSPL 2, RSPL 3, RSPL 4 and RSPL 5 respectively. Water was mixed with each of the RSPL (1-5) in three different proportions of 50:50, 60:40 and 70:30. The mixtures were each put into polythene bags, tied and kept in metal drums for treatment periods of 7, 14 and 21 days respectively. After each treatment period, the samples were dried under shade before used.

Experimental design

The design of the trial was a 5x3x3 factorial experiment triplicated three times 15.

Laboratory analysis

Samples of the treated rice straw were determined for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and ash according to 16 methods. The samples of the rice straw were also subjected to bacteriological study for pathogenic microorganisms[17].

Statistical analysis

The data collected was subjected to analysis of variance (ANOVA) and means separated by Duncan's multiple range tests 18 using the General Linear Model (GLM) in SPSS for windows[19].

Results

Table 1 shows the effect of ratio of rice straw (RS) to poultry litter (PL) on the nutrient composition and pathogen profile of the treated straw. The RS: PL ratio significantly (P<0.05) influenced the colony forming unit per gram (CFU/g). The ratio 5:5 and 6:4 had

Table 1: Effect of ratio of rice straw to poultry litter (RS: PL) on nutrient composition (% DM basis) and microbial flora of the treated rice straw.

Composition	RS:PL					SEM	LS
	5:5	6:4	7:3	8:2	9:1		
DM	90.65	91.35	91.81	91.50	91.20	2.77	NS
CP	13.46	12.49	11.83	12.09	10.99	1.77	NS
CF	23.39	23.67	23.91	23.92	24.83	1.27	NS
EE	1.42	1.39	1.37	1.51	1.22	3.09	NS
Ash	11.66	11.60	11.25	11.45	11.20	0.45	NS
CFU/g	$2.90x10^{6a}$	$2.36x10^{5a}$	$6.13x10^{3b}$	$1.14x10^{3b}$	$6.0x10^{2c}$	1.21	*

Means within the same row with different superscripts are significantly different.
*P<0.05; SEM= Standard error of mean; LS= Level of significance; NS= Not significant; DM= Dry matter; CP= Crude protein; CF= Crude fibre; EE= Ether extract; CFU/g= Colony forming unit per gram; RS: PL= Ratio of rice straw to poultry litter.

Table 2: Effect of ratio of water (WT) to rice straw-poultry litter (RSPL) mixture on nutrient composition (% DM basis) and microbial flora of rice straw.

Composition	WT:RSPL			SEM	LS
	50:50	60:40	70:30		
DM	91.64^{a}	90.73^{b}	81.54^{c}	2.77	*
CP	12.35	12.13	12.04	1.77	NS
CF	24.23	23.83	23.76	1.27	NS
EE	1.37	1.39	1.36	3.09	NS
Ash	11.54	11.45	11.30	0.45	NS
CFU/g	$6.27x10^{5}$	$8.48x10^{5}$	$4.11x10^{5}$	0.85	NS

Means within the same row with different superscripts are significantly different.
*P<0.05; SEM= Standard error of mean; LS= Level of significance; NS= Not significant; DM= Dry matter; CP= Crude protein; CF= Crude fibre; EE= Ether extract; CFU/g= Colony forming unit per gram; WT: RSPL= Ratio of water to rice straw- poultry litter mixture.

Table 3: Effect of days of treatment of rice straw-poultry litter mixture on nutrient composition (% DM basis) and microbial flora of rice straw.

Composition	Days of treatment			SEM	LS
	7	14	21		
DM	91.57	91.29	91.04	2.77	NS
CP	11.50^{b}	11.96^{b}	13.06^{a}	1.77	*
CF	24.35^{a}	24.13^{a}	23.25^{b}	1.27	*
EE	1.29^{a}	1.36^{b}	1.50^{a}	3.09	*
Ash	11.24	11.51	11.55	0.45	NS
CFU/g	$1.39x10^{6a}$	$4.31x10^{3b}$	$6.25x10^{4c}$	0.84	*

Means within the same row with different superscripts are significantly different.
*P<0.05; SEM= Standard error of mean; LS= Level of significance; NS= Not significant; DM= Dry matter; CP= Crude protein; CF= Crude fibre; EE= Ether extract; CFU/g= Colony forming unit per gram;

similar CFU/g; 2.90 x 106 and 2.36 x 106 for ratios 5:5 and 6:4 respectively. The ratio 9:1 recorded the lowest CFU/g of 6.9 x 102. The ratio 7:3 and 8:2 recorded similar CFU/g values. The other components, DM, CP, CF, EE and ash were not affected by ratio.

The effect of ratio of water (WT) to rice straw-poultry litter (RSPL) mixture (WT: RSPL) on nutrient composition and CFU/g of the treated rice straw is shown in Table 2. The CP, CF, EE, ash and CFU/g were not influenced by the ratio WT: RSPL with the exception of DM. The DM value declined with increase in WT and a corresponding decrease in RSPL. The ratio 70:30 recorded the lowest DM of 81.54%.

Day of treatment of rice straw significantly (P<0.05) affected the CP, CF, EE and CFU/g components of the treated straw (Table 3). The 21 days treatment period recorded the highest CP (13.06%), EE (1.50%) and lowest CF (23.25%) CFU/g (6.25 x 104) irrespective of mixture. The 7 and 14 days treatment periods recorded similar CP and CF values whereas, the 7 days treatment period recorded the highest CFU/g (1.39 x 106).

Table 4 shows the effect of days of treatment on some pathogenic microorganisms isolated from the treated rice straw. *Staphylococcus spp*. population was significantly (P<0.01) affected by days

Table 4: Effect of days of treatment on microorganisms isolated from the treated rice straw.

Microbes	Days of treatment			SEM	LS
	7	14	21		
Bacillus spp.	$1.17x10^{3}$	$3.11x10^{3}$	$1.42x10^{2}$	1.93	NS
Staphylococcus spp.	$2.02x10^{7a}$	$3.33x10^{6a}$	$2.32x10^{3b}$	7.48	**
Staphylococcus aureus	$1.73x10^{4a}$	$3.62x10^{6a}$	$2.34x10^{3b}$	2.65	*
Escherichia coli	$6.42x10^{5a}$	$2.63x10^{3b}$	$4.11x10^{2b}$	2.16	*
Micrococcus spp.	$1.15x10^{3}$	$1.76x10^{2}$	$2.63x10^{2}$	0.67	NS
Streptococcus spp.	$1.77x10^{3}$	$6.02x10^{2}$	$4.24x10^{3}$	0.22	NS

Means within the same row with different superscripts are significantly different.
*P<0.05; **P<0.01; SEM= Standard error of mean; LS= Level of significance; NS= Not significant;

Table 5: Interaction effect of TRT x RAT on nutrient composition (% DM basis) and microbial flora.

TRTxRAT	DM	CP	CF	EE	Ash	CFU/g
1 x 1	91.27	14.51^{a}	24.27	1.29	11.46	2.21x10^{5a}
1 x 2	89.64	13.67^{b}	23.21	1.49	11.47	1.64x10^{4b}
1 x 3	91.06	13.51^{b}	22.68	1.47	12.05	3.19x10^{3b,c}
2 x 1	91.66	12.08^{d}	24.08	1.46	11.37	2.05x10^{4b}
2 x 2	90.84	12.54^{c}	23.41	1.34	11.63	1.76x10^{3b,c}
2 x 3	91.55	12.88^{c}	23.51	1.37	11.79	1.36x10^{2c}
3 x 1	91.74	11.35^{e}	24.67	1.25	11.18	4.16x10^{3b,c}
3 x 2	92.08	11.83d	23.72	1.46	11.29	3.24x10^{4b}
3 x 3	91.59	12.31^{c}	23.33	1.41	11.30	1.16x10^{2c}
4 x 1	91.26	12.87^{c}	23.61	1.55	11.29	5.11x10^{2c}
4 x 2	91.21	11.70^{d}	23.98	1.52	11.49	6.04x10^{3b,c}
4 x 3	92.04	11.70^{d}	24.16	1.45	11.58	1.34x10^{2c}
5 x 1	91.77	11.73^{d}	24.54	1.26	11.22	5.02x10^{2c}
5 x 2	89.87	10.91^{e}	24.84	1.17	11.38	6.34x10^{3b,c}
5 x 3	91.95	10.32^{e,f}	25.12	1.24	11.00	1.86x10^{2c}
SEM	0.45	5.82	0.41	0.07	0.33	2.09

Means within the same column with different superscripts are significantly different.
*P<0.05; SEM= Standard error of mean; NS= Not significant; DM= Dry matter; CP= Crude protein; CF= Crude fibre; EE= Ether extract; CFU/g =Colony forming unit per gram; TRT= 1-5; RAT= 1-3.
TRT1= 5:5, 2=6:4,3=7:3,4=8:2,5=9:1
RAT1= 50:50, 2= 60:40, 3= 70:30.

Table 6: Interaction effect of TRT x DAY on nutrient composition (% DM basis) and microbial flora.

TRTxRAT	DM	CP	CF	EE	Ash	CFU/g
1 x 1	91.07	13.22	23.17	1.33	11.32	1.38x10^{4a}
1 x 2	90.09	13.49	23.42	1.45	11.94	4.05x10^{2c}
1 x 3	90.81	13.68	23.57	1.47	11.71	1.06x10^{2c}
2 x 1	91.59	11.87	24.21	1.29	11.59	2.11x10^{5a}
2 x 2	91.02	11.67	24.13	1.34	11.45	3.13x10^{3b}
2 x 3	91.45	13.95	22.65	1.54	11.75	7.05x10^{2c}
3 x 1	91.98	10.59	24.52	1.28	11.05	3.32x10^{4a}
3 x 2	91.73	11.68	24.11	1.32	11.35	1.47x10^{3b}
3 x 3	91.71	13.21	23.09	1.51	11.37	1.86x10^{2c}
4 x 1	91.75	11.39	23.35	1.45	11.04	3.04x10^{3b}
4 x 2	91.74	11.84	24.15	1.47	11.71	1.77x10^{2c}
4 x 3	91.02	13.06	23.26	1.59	11.59	3.47x10^{3b}
5 x 1	91.47	10.42	25.49	1.06	11.21	1.88x10^{3b}
5 x 2	91.92	11.13	24.85	1.19	11.08	4.65x10^{2c}
5 x 3	90.20	11.41	24.16	1.40	11.31	1.66x10^{2c}
SEM	0.38	0.42	0.33	0.04	0.15	1.78

Means within the same column with different superscripts are significantly different.
*P<0.05; SEM= Standard error of mean; NS= Not significant; DM= Dry matter; CP= Crude protein; CF= Crude fibre; EE= Ether extract; CFU/g= Colony forming unit per gram; TRT= 1-5; DAY= 1-3, TRT1= 5:5, 2=6:4, 3=7:3, 4=8:2, 5=9:1

of straw treatment. The population was lowest for the 21 days treatment (2.32 x 103). *Staphylococcus aureus* and *Escherichia coli* population were also affected (P<0.05) by days of treatment. However, there was a general reduction in the microbial population with days of treatment irrespective of isolate.

The interaction effects of TRT x RAT, TRT x DAY and RAT x DAY on the nutrient composition and pathogenic microbial flora is shown in Tables 5, 6 and 7. The TRT x RAT interaction influenced (P<0.05) the CP and CFU/g contents of the straw. The CP and CFU/g ranged between 10.32-14.51% and 1.16 x 102- 2.21 x 106 respectively. The treatment 5:5 and ratio 50:50 (i.e. TRT x RAT; 1:1) recorded the highest CP (14.51%). However, all the treatments (5:5) recorded superior CP values irrespective of ratio (Table 5). The CFU/g values were quite variable

Table 7: Interaction effect of RAT x DAY on nutrient composition (% DM basis) and microbial flora.

RATxDAY	DM	CP	CF	EE	Ash	CFU/g
1 x 1	91.70	11.15	24.85	1.21	11.27	$4.63x10^{4a}$
1 x 2	91.66	12.25	24.07	1.39	11.38	$3.26x10^{4a}$
1 x 3	91.26	12.74	23.79	1.48	11.26	$1.79x10^{3b}$
2 x 1	91.16	11.44	24.33	1.31	11.01	$6.16x10^{3b}$
2 x 2	90.44	11.32	24.34	1.28	11.53	$1.64x10^{3b}$
2 x 3	90.58	13.57	22.82	1.59	11.81	$8.10x10^{2c}$
3 x 1	91.85	11.84	23.86	1.33	11.44	$4.82x10^{4a}$
3 x 2	91.80	12.32	23.99	1.39	11.61	$1.98x10^{3b}$
3 x 3	91.27	12.88	23.44	1.45	11.57	$5.05x10^{2c}$
SEM	0.72	0.68	0.52	0.10	0.28	2.48

Means within the same column with different superscripts are significantly different.
*P<0.05; SEM= Standard error of mean; DM= Dry matter; CP= Crude protein; CF= Crude fibre; EE= Ether extract; CFU/g =Colony forming unit per gram; RAT= 1-3; DAY= 1-3, RAT1= 50:50, 2= 60:40, 3= 70:30; DAY1= 7, 2= 14, 3=21.

although relatively high for TRT 5:5. The interaction of TRT x DAY did not affect the DM, CP, CF, EE and ash contents of the treated straw. However, the CFU/g was significantly (P<0.05) influenced with values between 1.06 x 102 and 2.11 x 105. A general decline was observed in CFU/g from treatments 1 to 5. Day 3 (21 days) consistently recorded the lowest CFU/g across the treatments (Table 6). Table 7 shows the interaction effects of RAT x DAY on the composition of the treated straw. The CFU/g was the only factor influenced (P<0.05) by the interaction with values ranging between 5.05 x 102 and 4.63 x 104. There was a low CFU/g for 21 days treatment across ratios.

The interactions of TRT x RAT x DAY as shown in Table 8, did not influence any of the parameters measured. However, the CF and CFU/g values were generally low for the 21 days treatment across the interactions. Also, the CP contents were comparatively higher.

Discussion

The high CFU/g observed for the ratio 5:5 may be attributed to the large quantity of poultry litter used in the treatment of the rice straw. This result confirms earlier report[20] that poultry litter is a carrier of many pathogenic microorganisms, and that processing of the litter either by ensilage alone or with other materials can nullify the risk of spreading diseases.

The reduced DM observed in this study may be due to the large amount of water mixed with the rice straw-poultry litter mixture. This low DM could have influenced the relatively low nutrient contents of the treated material.

The increase in CP of the treated straw especially for the 21 days (13.06%) confirms earlier report[21] that day of ensilage of urea-treated roughage leads to an increase in CP. However, the CP value for the 21 days treatment period is higher than values reported for 5, 15 and 25 days

Table 8: Interaction effect of TRT x RAT x DAY on nutrient composition (% DM basis) and microbial flora.

TRTxRATxDAY	DM	CP	CF	EE	Ash	CFU/g
1x1x1	91.16	11.31	24.39	1.14	11.06	$1.15x10^{5}$
1x1x2	91.71	12.08	24.33	1.31	11.67	$3.42x10^{4}$
1x1x3	90.93	13.22	24.00	1.41	11.65	$1.74x10^{4}$
1x2x1	91.16	12.75	23.43	1.27	10.88	$4.76x10^{4}$
1x2x2	87.04	12.90	23.92	1.43	11.43	$9.03x10^{3}$
1x2x3	90.72	12.37	22.28	1.76	12.09	$1.93x10^{4}$
1x3x1	90.89	15.60	21.68	1.58	12.03	$2.33x10^{4}$
1x3x2	91.51	15.49	22.02	1.59	12.73	$1.46x10^{4}$
1x3x3	90.79	16.45	24.35	1.34	11.38	$7.71x10^{3}$
2x1x1	91.84	11.55	24.37	1.39	11.55	$2.36x10^{4}$
2x1x2	91.59	11.78	24.44	1.46	11.06	$1.75x10^{3}$
2x1x3	91.56	12.90	23.43	1.53	11.50	$3.21x10^{3}$
2x2x1	91.38	12.23	23.97	1.20	11.36	$1.39x10^{3}$
2x2x2	89.69	10.93	24.29	1.24	11.79	3.32x103
2x2x3	91.43	14.45	21.98	1.58	11.75	$1.63x10^{2}$
2x3x1	91.54	11.83	24.31	1.30	11.87	$4.28x10^{2}$
2x3x2	91.76	12.30	23.67	1.31	11.49	$1.76x10^{3}$
2x3x3	91.35	14.49	22.56	1.51	11.99	$1.08x10^{2}$
3x1x1	91.55	9.59	26.30	0.94	10.97	$1.06x10^{3}$
3x1x2	92.09	11.92	24.11	1.34	11.13	$1.77x10^{2}$
3x1x3	91.59	12.52	23.59	1.45	11.43	$7.32x10^{2}$
3x2x1	92.13	10.28	24.43	1.58	10.92	$1.13x10^{2}$
3x2x2	91.60	11.03	24.25	1.21	11.43	$2.36x10^{3}$
3x2x3	92.52	14.17	22.47	1.59	11.51	$1.23x10^{4}$
3x3x1	92.26	11.89	22.82	1.33	11.26	$3.21x10^{3}$
3x3x2	91.49	12.09	23.96	1.40	11.49	$1.74x10^{2}$
3x3x3	91.02	12.95	23.22	1.49	11.16	$7.67x10^{2}$
4x1x1	92.56	12.28	24.03	1.51	11.12	$1.66x10^{3}$
4x1x2	91.44	13.29	23.47	1.56	9.88	$2.34x10^{3}$
4x1x3	89.79	13.04	23.33	1.59	10.84	$5.78x10^{4}$
4x2x1	90.59	11.79	24.09	1.49	11.07	$1.24x10^{3}$
4x2x2	91.34	11.10	24.25	1.47	9.72	$2.74x10^{3}$
4x2x3	91.69	12.22	23.62	1.59	11.67	$3.23x10^{3}$
4x3x1	92.09	10.09	24.93	1.35	10.92	$1.18x10^{2}$
4x3x2	92.46	11.11	24.73	1.39	10.54	$3.21x10^{2}$
4x3x3	91.58	13.91	22.83	1.59	12.26	$1.98x10^{2}$
5x1x1	89.41	11.00	25.14	1.07	9.66	$1.33x10^{3}$
5x1x2	90.47	12.15	23.99	1.30	11.15	$6.10x10^{2}$
5x1x3	86.43	12.02	24.48	1.39	9.86	$2.32x10^{2}$
5x2x1	90.55	10.44	25.75	1.04	9.84	$1.62x10^{3}$
5x2x2	92.51	10.64	25.00	1.06	10.27	$3.24x10^{2}$
5x2x3	86.56	11.65	23.77	1.41	10.01	$1.91x10^{2}$
5x3x1	92.45	9.81	25.57	1.07	11.13	$9.02x10^{2}$
5x3x2	89.78	10.59	25.55	1.24	9.91	$8.33x10^{3}$
5x3x3	91.62	10.58	24.24	1.40	11.07	$1.79x10^{2}$
SEM	0.35	0.27	0.19	0.04	0.11	3.32

SEM= Standard error of mean; DM= Dry matter; CP= Crude protein; CF= Crude fibre; EE= Ether extract; CFU/g Colony forming unit per gram;
TRT= 1-5; RAT= 1-3; DAY= 1-3.
TRT1= 5:5, 2=6:4, 3=7:3, 4=8:2, 5=9:1.
RAT1= 50:50, 2= 60:40, 3= 70:30.
DAY1= 7, 2= 14, 3= 21.

urea-treated rice straw; 12.86, 10.50 and 12.10% respectively[22].

The reduction in CF level with days of treatment could be attributed to the breakdown of the lignocelluloses bond in the straw by the ammonia released from the hydrolysis of uric acid in the poultry litter. Similar observation has been made[22].

The reduced CFU/g with days of treatment agrees with earlier reports[4, 23, 24] that ensiling poultry litter alone or with green

or dry roughage for several days makes the treated material sterile so far as pathogenic microbes are concerned.

The presence of microorganisms in poultry excreta is in agreement with earlier reports[25, 26] that poultry litter or excreta are carriers of several pathogens. However, the treatment of the rice straw by ensiling with poultry litter reduced the microbial population of the treated material. This result confirms reports[27, 28] that ensiling poultry waste is the most feasible and effective method of destroying pathogens thereby nullifying the risk of spreading animal diseases when fed to livestock.

The high CP recorded for the interaction of TRT x RAT especially for TRT 5:5, may be attributed to the large amount of poultry litter used in the treatment process. Enough ammonia gas may have been hydrolyses from the uric acid in the poultry litter which must have been partly used to breakdown the lignin bond and partly located within the treated material. This observation confirms earlier report[12] that ensiling poultry waste does not only prevent CP losses, but also converts part of the non-protein nitrogen (NPN) to true protein.

The CFU/g though quite variable across the two-way interactions, was relatively high were large quantities of poultry litter was used. This confirms earlier reports[20, 26]. However, interaction of RAT x DAY showed reduction in microbial population especially at 21 day. This implies that treating of the material for longer periods makes it safer for use in feeding to livestock[23, 29].

The three-way interaction of TRT x RAT x DAY recorded high CP (between 9.59-16.45%) compared to values obtained for urea-treated rice straw (5.88-12.86%), sorghum stover (3.69-11.38%) and maize stover (4.69-10.06%) for untreated to 25 days treatment[22]. This high CP content may be due to the hydrolysis of uric acid to ammonia in addition to, microbes that might have lysed and possibly spilled feed in the treated litter. The low CFU/g in the interaction confirms earlier reports[4, 30].

Conclusion

The result of this study has shown that ensiling rice straw with poultry litter can improve the feeding value of the treated material by increasing crude protein and reducing crude fibre. The poultry litter is also made safe by the reduction in population of pathogenic microbes. Poultry litter can therefore be a very good alternative to urea fertilizer in ammoniation of crop residues for livestock feeding. However, the proportion of crop residue to poultry litter and duration of ensiling is critical for effective treatment. Equal proportions of crop residue to poultry litter with an equal proportion of water and, a minimum of 21 days (3 weeks) treatment period is required for effectively treating fibrous crop residues and it is safe for feeding animals. In addition, treating fibrous crop residues with poultry litter is a suitable means of recycling poultry litter thereby reducing or preventing the problem of litter disposal and environmental pollution in intensive poultry production systems.

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EVALUATION OF PROCESSED VELVET BEANS *(MUCUNA PRURIENS)* MEAL IN THE DIET OF LAYING HENS.

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Etude comparative des Performances de trois Espèces d'Escargots Comestibles dans un Système Intensif d'élevage au Cameroun

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EVALUATION DU REGIME A BASE DE POIS MASCATES TRAITES *(MUCUNA PRURIENS)* CHEZ LES PONDEUSES

Sommaire

Les effets de la complémentation alimentaire à base de pois *Mucuna* de 20, 25 et 30% craqué, trempé et cuits (CSCM) sur la production d'œufs, la qualité d'œuf et le poids des organes ont été étudiés chez des pondeuses âgées 12 mois. Les pois traités ont été ajoutés à la ration des pondeuses à base du maïs et du soja, nourris *ad libitum* pour une période de 12 semaines. Tous les régimes alimentaires à base Mucuna ont conduit aux réductions lineaires de la production chez les pondeuses – production journalière chez les poules nourries avec du CSCM à 20, 25, et 30% ont connu une réduction respective de 9,3, 13,9 et 17,5% par rapport aux poules témoins (0% CSCM). Quant au poids des œufs, il a augmenté de manière proportionnelle (P<0,05) à l'inclusion du CSCM de 25% (66.7g) et 30% (68.2g) contre 61.3g pour le contrôle. La consommation d'aliments a augmenté de manière significative (P<0,05) dans les groupes alimentaires CSCM 20% et 25%. Le rapport aliments consommés pour une douzaine d'œufs produite était meilleure (P<0,05) dans le groupe de contrôle (1,74) que dans le groupe alimentaire *Mucuna* 30% (2.24). Au niveau d'inclusion alimentaire de 20%, la CSCM a provoqué une meilleure production d'œufs et un rapport meilleur que dans les groupes nourris à 25 et 30%., Le poids des poumons a été plus bas (P<0,05) dans les groupes recevant du CSCM . Il est conclu que l'inclusion alimentaire de la CSCM dans l'alimentation des pondeuses peut provoquer une diminution dans la production journalière, mais conduire à une production des oeufs plus lourds.

Mots-clés: Production d'oeufs, qualité d'œuf, poids d'organes, traitement, pois mascates.

Summary

The effects of dietary additions of 20, 25 and30% cracked soaked and cooked *Mucuna* (CSCM) beans on egg production, egg quality and organ weight were studied using 12-month in –lay laying hens. The processed beans were added to layers ration at the expense of maize and soybean meal to meet or exceed the nutrient requirements and were fed *ad libitum* for a 12-week period. All *Mucuna* based diets caused graded reductions in hen – day production with birds fed 20, 25, and 30% CSCM diets having 9.3, 13.9 and 17.5% reductions from control respectively. Egg weight increased with increasing dietary inclusion of the meal with birds fed 25% (66.7g) and 30% (68.2g) CSCM having a significantly (P<0.05) heavier eggs than the control (61.3g). Feed consumption increased significantly (P<0.05) in the 20 and 25% CSCM diet groups.

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Feed/dozen egg produced was significantly (P<0.05) better in the control (1.74) diet than in the 30% *Mucuna* (2.24) diet group. At 20% dietary inclusion level, CSCM promoted a much better egg production and efficiency than 25 and 30% but the value was significantly less than control. The haugh unit, shell thickness, albumen and yolk index, and egg size from birds fed *Mucuna* diets compared favorably with the control. The lungs weights were significantly (P<0.05) lower in the *Mucuna* diet groups than in the control. It is concluded that dietary inclusion of CSCM in the diet of laying hens caused a decreased in hen-day production but produced heavier eggs with increasing dietary levels.

Key words: Egg production, egg quality; organ weights, processing, velvet bean.

Introductions

Velvet bean plant *(Mucuna pruriens)* is an important cover crop (or green manure crop) in many parts of the world especially among subsistence farmers[1]. It produces prolific quantities of bean or seeds. The raw bean contains about 2370-kcal/kg energy[2] and 32% crude protein that have a relatively good amino acid profile[3]. The use of the bean in poultry feeding is one of the best ways of exploiting its agronomic and nutritional potentials. Its use could also help in reducing the over-dependence on the conventional protein supplements notably; soybean (Glycine max) and groundnut (Arachis hypogea) and hence reduce the ever-increasing price of concentrate feeds for non-ruminant animals.[4, 5, 6].

However, the use of unprocessed raw velvet beans in non-ruminant diets showed reduced feed intake, loss of weight and mortality in pigs[4, 5, 6] and depressed growth and egg production in poultry[7,8,9,2]. Its use also increased the heart, proventricular, gizzard and pancreas weights as well as the lengths of the small and large intestines, and ceca in broilers[10].

Among the possible causes of the variability in performance and organ weights were the presence of ant-nutritional factors which included: tannins, cyanide, phenols, lectins, amylase inhibitors, anti-trypsin factors, dihydroxy phenylalanine *(L. Dopa)*[11, 12], anticoagulants[13], analgesic, anti-pyretic and anti-inflammatory factors[14] and others[8]. Various authors[2, 12, 8,15] reported partial or total destruction of the anti- nutritional factors in the bean by heat treatment methods. Moist or dry heat treatment of the bean[9, 2, 16], soaking before cooking or roasting[17, 18, 19, 20] and roasting with urea[21] only gave indications of partial detoxification hence its dietary inclusion levels could not exceed 10-20% for broilers. Organ weights were also report to have been partially or wholly restored to the control levels[9,22]. Broilers fed 12 and 18% roasted velvet bean but not 6% had increased liver and spleen weights but not heart or gizzard weights[16].

However, data on egg production is sketchy. Laying hens fed 6% of either raw, wet autoclaved or roasted (in sand over fire) velvet bean from 5% production to 30 weeks of lay had reduced feed intake only with the raw and non of the treatments affected egg size as measured by weight, length or width[16]. The egg yolk index of the eggs was not affected by any of the treatments.

Recent studies have, however, shown that 25 and 40% CSCM were tolerated by broilers[23] and pigs[24] respectively, without any detrimental effect on performance. The beans so processed have not been tried on laying

hens. The trial herein reported was therefore designed to examine the effect of CSCM on egg production, egg quality and organ weight of laying hens.

Materials and method

Velvet bean and processing.

Dried velvet bean *(Mucuna pruriens)* seeds with black coat colour were harvested from the Teaching and Research Farm of the School of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Nigeria where this experiment was conducted. Owerri is located in the southeast Agro ecological zone of Nigeria. *Mucuna* beans were cracked into 2-4 parts/ seed using an ASKO All electric grinding mill. The cracked beans were soaked in water at room temperature for 48hours with the water covering the seed in the vat. After soaking, the beans were then rinsed with

Table 1: Ingredient composition of layers diets (g/100g DM).

Ingredients	**Control 0.0%**	**CSCM 20.0%**	**CSCM 25.0%**	**CSCM 30.0%**
Maize	55.00	46.00	43.00	40.00
Mucuna seed meal	-	20.00	25.00	30.00
Soya bean meal	17.00	6.00	4.00	2.00
Wheat offal	9.00	9.00	9.00	9.00
Palm kernel cake	2.00	2.00	2.00	2.00
Fish meal	3.00	3.00	3.00	3.00
Blood meal	3.00	3.00	3.00	3.00
Bone meal	6.00	6.00	6.00	6.00
Oyster shell	4.00	4.00	4.00	4.00
L-lysine	0. 25	0.25	0.25	0.25
L-methionine	0.25	0.25	0.25	0.25
Vit. Premix*	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis (%)				
Crude protein (CP)	18.38	18.41	18.68	18.94
Crude fibre (CF)	4.03	3.51	3.42	3.32
Ether extract (EE)	3.76	3.69	3.67	3.64
Calcium (Ca)	3.82	4.00	4.05	4.09
Phosphorus (P)	1.45	1.48	1.50	1.51
ME (Kcal/kg)	2655.98	2760.90	2804.54	2848.18

**To provide the following per kg of feed.*

Vit. A, 10,000iu; Vit D3, 1500iu; Vit. E, 3iu, Vit. K, 2mg; riboff, 3mg; pantothenic acid, 6mg; niacin, 15mg; choline, 5mg; Vit. B12, 0.08mg; folic acid, 4mg; Mn, 8mg; Zn, 0.5mg; Iodine, 1.0mg; Co, 1.2mg; Cu, 10mg; Fe, 20mg.

clean fresh water and drained out. The water-drained beans were cooked in water for 60 minutes. After the boiling, the beans were sun dried on a concrete slab until they contained less than 13% moisture. The dried seeds were ground into meal using ASKO All electric grinding mill to produce cracked soaked and cooked Mucuna meal (CSCM).

Experimental diets

Four experimental diets were evaluated. The processed velvet beans were added to commercial type control diet at levels of 20, 25 and 30%. The composition of the diets is shown in table 1. The diets contained levels of nutrients equal to or exceeding those described by the National Research Council[26]. Twenty, 25 and 30% CSCM were added to the control diet partially replacing maize and soybean meal. All ingredients, including processed Mucuna were mixed manually and fed as a meal for 12 weeks.

Experimental birds and design

Twelve months in-lay Shika brown laying hens were used in the study. They were housed in two- tier battery cage, in an open – sided, naturally ventilated house. The birds were divided into four groups of 20 each. Four cage units were assigned to each of the four treatments: Control and 20, 25 and 30% CSCM. Each unit of the cage contained 2 birds. The birds had 12- hours of daylight. The room temperature varied from 25^0C at night to 33^0C during the day, while relative humidity ranged from 75 – 86.6%. Feed and water were provided ad libitum. The birds were weighted at the beginning of the experiment and weekly there after, while feed intake was weighed daily. Eggs were collected twice daily (9.00am and 3.00pm) and weighed.

Five eggs from daily collections from each treatment were randomly selected and evaluated daily for haugh unit, shell thickness, yolk index and albumen index. Organ weights (heart, liver, kidney, lungs, and gizzard) were determined from 25% of the population of each treatment. The birds were killed, eviscerated and the weights of heart, liver, lungs and gizzard measured and expressed as percentage of live weight. A completely randomized deigned was used on the study.

Data Analysis

Data were analyzed using one-way analysis of variance (ANOVA). The Duncan's Multiple Range Test was used to test differences between means[25]. Statement of statistical significance were based on $P<0.05$.

Result

Table 2 shows the effect of dietary inclusion of CSCM on laying performance and egg quality. Hen-day production decreased linearly as the dietary level of CSCM was increased ($P<0.05$). Birds fed 20, 25 and 30% CSCM had 9.3, 13.9 and 17.5% reductions in egg production, respectively. The control diet was significantly ($P<0.05$) better than Mucuna diet groups in hen-day egg production. Birds fed 20% CSCM diet had a significantly ($P<0.0$) better hen-day egg production than those fed 30% CSCM diet.

The result indicated a significantly ($P<0.05$) higher body weight change among birds fed CSCM diets. Birds fed 20 (160g) and 25% (150%) CSCM diets had a significantly ($P<0.05$) higher body weight changes than the control (130g) and 30% (120g) diet groups. Egg weights increased linearly as the dietary level of

Table 2: Effect of processed mucuna seed meal on the performance and egg quality characteristics of laying hens

Parameters	Control 0.0%	CSCM 20.0%	CSCM 25.0%	CSCM 30.0%	SEM
Hen-day production (%)	77.80[a]	68.53[a]	63.86[b]	60.32[b]	2.00
Body weight change (g)	130.00[b]	160.00[a]	150.00[a]	120.00[b]	5.13
Average egg weights (g)	61.31[b]	65.22[ab]	66.65[a]	68.19[a]	1.48
Feed intake (g/b/d)	112.68[b]	116.05[a]	115.81[a]	112.66[b]	0.94
Kg feed/kg egg.	1.84[a]	1.78[a]	1.74[ab]	1.65[b]	0.04
Haugh unit (HU)	78.03	80.69	78.18	79.93	0.66
Shell thickness (mm)	0.39	0.36	0.38	0.36	0.01
Yolk index	0.39	0.40	0.41	0.40	0.004
Albumen index	0.05	0.05	0.05	0.05	0.00
Horizontal circumference (cm)	13.93[b]	14.17[ab]	14.33[a]	14.07[b]	0.08
Oblong circumference (cm)	16.16[ab]	16.56[a]	16.63[a]	15.93[b]	0.17

ab: means within a row with difference superscripts differ significantly (p<.05)

CSCM was increased (P<0.05). Birds fed 20, 25 and 30% CSCM diets laid eggs that weighed 61.3, 65.2, 65.7 and 68.2g respectively. The control diet group had a significantly (P<0.05) lower egg weight than 25 and 30% CSCM diet groups.

Feed intake value were significantly (P<0.05) higher in the 20 and 25% diet groups than the control or 30% group. Generally both the control and 30% diet groups had lower feed intakes (112.7, 112.7g) than the 20% (116.1g) or 25% (115.8g) diets groups. Feed/dozen egg lay decreased as the dietary level of CSCM was increased (P<0.05).Birds fed the control, 20, 25 and 30% CSCM diets had 1.74, 2.03, 2.18 and 2.24 feed/dozen egg ratios respectively. All the *Mucuna* diets groups, except 30% compared statistically in feed/ dozen egg ratio with the control. The haugh unit, shell thickness, yolk index and albumen index values were similar among treatments. The egg size as measured by the horizontal and oblong circumferences were significantly different across treatments (P<0.05). The largest egg horizontal and oblong circumference values were obtained from birds fed 25% CSCM (14.3; 16.6cm) diet. Birds fed 20 and 30% CSCM diets had a statistically (P<0.05) similar egg horizontal and oblong circumference values with the control.

Table 3 shows the effects of CSCM on internal organs of laying hens. Both the heart, gizzard and lung weights were significantly (P<0.05) affected by the treatments. Birds fed 25% CSCM diet had a significantly (P<0.05) heavier heart weight (0.58%) than those fed 20 and 30% diets. Similarly birds fed 20% diet had a significantly (P<0.05) heavier gizzard weight (2.64%) than those fed 25% or 30% diet. Birds on the control diet had a statistically similar percent heart (0.54%) and gizzard (2.44%) weights with the CSCM diet groups. The percent lungs weight was significantly (P<0.05) higher among the control (0.046%) than the 20 and 25% velvet bean diet groups. Birds on 30% CSCM diet had a statistically similar percentage lungs weight (0.44%) with the

Table 3: Effect of processed *Mucuna* seed meals on the visceral organ weights of laying hens.

Parameters:	Control 0.0%	CSCM 20.0%	CSCM 25.0%	CSCM 30.0%	SEM
Heart	0.54^{ab}	0.15^{b}	0.58^{a}	0.51^{b}	0.02
Liver	1.80	1.90	1.68	1.97	0.06
Kidney	0.22	0.22	0.22	0.25	0.01
Lungs	0.46^{a}	0.41^{b}	0.42^{b}	0.43^{ab}	0.01
Gizzard	2.44^{ab}	2.64^{a}	2.35^{b}	2.28^{b}	0.08

- *% of live weight*
- *a b: means within a row with different superscripts are significantly different (p<0.05).*

control. The percent liver and kidney weights were not significantly affected by the treatments.

Discussion

Little or no systematic research has been done on the nutritional quality of Mucuna pruriens for laying hens. Raw Mucuna pruriens at 5% dietary inclusion level caused a decreased in egg production[7]. It also decreased feed intake in layers at 6% dietary inclusion level[19]. Heat treatments[9, 2, 17, 18, 20] have been reported to only partially improve the nutritional value of the bean for broilers. It has been reported that processing partially or wholly restored the internal organ weights of broilers to normal with the control[16]. However, the method of processing that will produce optimal result in poultry has not been establishment.

It is a major objective of the experiment to investigate whether the beneficial effects of CSCM observed in broilers[23] and weaner pigs[6] could be reproduced in laying hens as well as the replacement value of the meal for maize and soybean. In the present study it was found that including any level of CSCM from 20 – 30% in the diet of laying hens from 12 months in lay through 12weeks of research caused progressive reductions in hen-day egg production with an extreme depression produced by feeding 30% CSCM, contrary to improve growth reported for broilers[23]. Part of this effect may be explained by the lack of knowledge about nutrient availability in the different diets especially amino acids in the processed velvet bean although the diets were nutritionally balanced based on the data available to meet or exceed the National Research Council[26] requirements for laying hens. However the nutritional value of processed velvet bean has not been studied extensively in laying hens. On the other hand, a large part of the negative effect of the beans on egg production is undoubtedly explained by one or more heat stable toxic factors present in the beans including *L. Dopa*[15, 27].

Our results show that feed intake is less severely affected by processed velvet bean than is egg production and only the 30% level reduced feed intake, though the value was similar to that of the control. It is important to note that the poor egg production rate shown by the hens was extremely not due to voluntary intake of hens fed with *Mucuna* based diets. Birds fed 20 and 25% CSCM consumed more feed. It is

therefore clear that the birds were consuming nutrients within or above their daily requirements but could not produce eggs in line with feed consumed. It is unclear why processed Mucuna diet had such improved palatability. Similar improved feed intake have been reported for laying hens fed 6% wet autoclaved or roasted velvet bean contrary to the depressed feed intake of the raw bean[16]. Apparently the major nutritional effect of CSCM is to inhibit the metabolic processes leading to egg production apart from an increased effect on appetite.

Interestingly egg weight increased with increasing dietary inclusion of CSCM contrary to its decreasing effect on egg production. Similar increase in egg weight has been reported in laying hens fed 6% raw or processed velvet bean[16]. It therefore appears that Mucuna bean has a property that enhances egg weights and feed intake at the expense of egg production.

Feed per dozen egg produced was markedly depressed by the 30% level of CSCM reflecting its depressive effect on egg production. Unfortunately data on laying hens in the literature is very sketchy and so far does not give much evidence to support these results. An interesting case of poor feed conversion ratio at higher dietary inclusion levels is the example of CSCM fed to starter broilers[23]. Dietary levels appear to be the most important factors affecting performance in the utilization of CSCM as a source of protein and energy to replace soybean and maize for laying hens. It was evident by comparing the egg production of hens fed 20% CSCM with those fed 25 and 30% (Table2) that 20% had less drastic effects on egg production. Other measures such as feed intake and feed conversion ratio were also better in laying hens fed 20% CSCM compared to 25 and 30% levels (Table 2).

The haugh units of eggs in the present study varied form 78.0 – 80.7. The values were above the cut-off level of 75 set for high quality fresh eggs (28), thus suggesting that dietary inclusion levels of CSCM did not adversely affect the freshness of eggs. The shell thickness, egg yolk index as well as egg albumen index were not affected by any of the treatments. The result on egg yolk index was in line with earlier reported on raw, wet autoclaved or roasted velvet beans fed to layers[16]; thus confirming the value of the bean in maintaining egg quality. The processed bean tended to increase the egg size as measured by the oblong and horizontal circumference especially at 25% dietary inclusion level. Earlier report on egg size[16] showed that egg size as measured by egg weight, length or width were not negatively affected by dietary inclusion of 6% raw, wet autoclaved or roasted velvet beans. The reason for the higher egg size and weight among the velvet bean diet groups is some how difficult to explain but may be attributed to a yet to be identified factor in the beans.

Slight differences were observed between the control and *Mucuna* diet groups in organ weights. Birds fed 25 and 20% CSCM diets had the highest heart (0.58%) and gizzard (2.64%) weights that compared to that of the control (0.54, 2.44%). Liver and kidney weights were unchanged while lungs weight decreased significantly at 20 and 25% dietary levels in spite of the fact that birds had a higher body weigh change (Table 2). An increase in organ weights would be expected as animals add more weight but the lungs weight did not follow this expectation at 20 and 25% dietary levels. The reason for the decrease in lung weights at these levels cannot be rightly explained

as birds fed 30% CSCM compared favorably with the control in lungs and other organ weights measured. Earlier investigation on broilers showed that heated velvet bean partially or wholly restored the internal organs to normal[10]. If that is the case it could be said that the different dietary inclusion levels of CSCM in the diet of laying hens had no adverse negative effect in the organs.

Conclusion

The inclusion of CSCM at 20 – 30% in the diet of laying hens caused graded reductions (9.3 – 17.5%) in egg production rate while egg weight (61.3 – 68.2) increased. Egg quality and organ weights were not adversely affected by the treatments. Of all the dietary level of CSCM used, 20% was the best and significantly superior to 30% in terms of egg production but the value was still significantly ($P<0.05$) lower than with the control diet. The most striking effect of the inclusion of CSCM regardless of the level was the increased egg weight and feed intake. The specific factor(s) in the processed been causing these increases at the expense of egg production is something that should be investigated. The use of up to 20% inclusion level of CSCM in the diet of laying hens should only be encouraged where costs and availability posses a problem to the use of maize or soybean meal. Cooking presoaked whole or cracked beans in local alkaline solution, a method that allowed for 30% inclusion in broiler's diet[24] should be explored in laying birds.

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COMPARATIVE PERFORMANCE OF THREE EDIBLE SNAIL SPECIES USING INTENSIVE CAGE HOUSING SYSTEM IN CAMEROON

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ETUDE COMPARATIVE DES PERFORMANCES DE TROIS ESPÈCES D'ESCARGOTS COMESTIBLES DANS UN SYSTÈME INTENSIF D'ÉLEVAGE AU CAMEROUN

Sommaire

Cette étude a été menée pour évaluer les performances de trois espèces d'escargots comestibles (*Archachatina marginata* – escargot géant, *Archachatina fulica* – *fulica* et *Archachatina archachatina* – petit escargot) dans des cages reproduisant les conditions de leur habitat naturel. Après 24 semaines d'étude, les escargots *fulica* et les petits escargots se sont multipliés plus vite que l'escargot géant. Bien qu'aucune différence significative n'ait été observée sur les poids vif du petit escargot de jardin et le *fulica*, ils étaient environ six fois plus élevés chez les escargots geants. La production d'oeufs était plus élevée (15 à 25 oeufs/couvée à la ponte) chez le petit escargot de jardin comparée à l'escargot géant (8 à 12 œufs/couvée à la ponte). Le taux d'éclosion d'oeufs s'est élevé à 100% pour l'escargot géant et un peu moins pour le petit escargot et le *fulica* (92,86%). La période d'incubation a varié de 21 à 23 jours pour le petit escargot à entre 25 et 28 jours pour l'escargot géant. Le poids des nouveau-nés a été le plus élevé (3,35 g) pour l'escargot géant et (1,07 g) pour le petit escargot et le *fulica*. Ces résultats montrent que l'élevage d'escargots en cage exige peu de travail et capitaux.

Mots clés: escargots, *Archachatina,* cages, système de logement, performance, Cameroun.

Summary

This study was undertaken to investigate the performance of three edible snail species *(Archachatina marginata*-giant land snail, *Archachatina fulica* – *"long mop"* and *Archachatina archachatina* – garden snail) using cages with conditions that mimicked their natural habitats. After 24 weeks of study, Archachatina fulica and *Archachatina archachatina* multiplied faster than *Archachatina marginata*-giant land snail. Although no significant differences were observed with respect to the live-weights of garden snail and *fulica,* average live weights were about six-fold higher for *Archachatina marginata*. Egg production was highest with (15-25 eggs/clutch at lay) for garden snail and least with the giant land snail (8-12 eggs/clutch at lay). Percentage hatchability of eggs was 100% for the giant land snail and slightly less for both the garden snail and *fulica* (92.86 %). The incubation period varied from 21 - 23 days for garden snail to 25 - 28 days for the giant land snail. The weight of hatchlings were highest (3.35 g) for giant land snail and (1.07 g) for the garden snail and *fulica*. These results show that snail rearing using cage housing system requires little labour and capital investments (US$ 50), to produce (US$ 500, 350 and 250) for garden snail, giant land snail and *fulica* respectively on a year-round harvest.

Key word: Snails, *Archachatina,* cages, housing system, performance, Cameroon.

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Introduction

In Cameroon, poverty affects 40.2% of the population and 85% recite in the rural areas employing close to 60% of the active population[1]. A large part of the population feeds mostly on bulky starchy food and being poor, are unable to satisfy their protein needs leading to poor nutrition with its attendant consequences. Snails are rich in protein and consumed by all classes of person and would serve as a solution to the problem of protein-energy malnutrition to the rural poor. However, their persistent scarcity and fluctuation of prices in Cameroon with higher price during the dry season is a cause for concerned. Presently the rate of production has not kept pace with demand as snails are consumed all over the world[2]. In Cameroon, production is still subsistence with most of the collection done from the wild and besides local consumption; a lot of the snails are processed and exported to Europe and America[3]. This has caused local shortages with price hikes for the little available to local consumers. Continuous search from the wild and subsistence level of production may not be sufficient to meet the current trend of human demands. This situation can be prevented through intensive snail production using different housing and feeding systems.

Additionally,[4] stressed that the situation can be prevented through proper management practices (i.e. adequate wetting, shading and mulching). Rearing of snails on a continuous basis that trans-pass the rainy and dry season through good management practices is another solution[5]. Snail farming as acknowledged[6] is well known in Europe, can also be developed in Africa using indigenous edible species such as *Archachatina archachatina, Archachatina marginata, Archachatina fulica, Limicolonta Spp* etc.

Basically, animal protein is obtained from meat, milk and eggs. Meat sources of conventional livestock are usually expensive for an average Cameroonian. Snails from the wild can be easily managed with little or no capital investment to boost and complement the protein intake of the people[7].Meanwhile[8] reported that snails are good sources of protein and that the protein content ranges between 16-18%. Snails will easily relish unconventional feeds like leaves, fruits, households and industrial remnants which are found in dust bins thus aids in biodegradation of these wastes. Snail rearing would be a good source of income and less capital is use than other livestock enterprises, and its return on investment is high because of low capital input.

Considering the potentials and prospects in the snail sector, it is but imperative that studies should be on the way for the establishment of a proper and sustainable snail industry in Cameroon and beyond. This will generate effective and optimal performance of the chosen species under intensive management systems. The objective of this study was to compare the performance of three edible snail species using the intensive cage housing system.

Materials and Methods

This study was carried out at the Regional Institute of Agricultural Research for Development, Ekona with an average annual rainfall of 2300mm, average temperature 24-25^{0}C and relative humidity of 90%.

Housing system

Cages of 1.5mx0.5m were used to

house 100 snails of each species.

Choice of soil and its preparation

Loamy soil prepared in the ratio of 3:1 i.e. 03 parts of soil to 01 part of sharp sand was used to fill the cages to a depth of 10cm. To this sandy-loamy soil, 1kg of oyster/bone meal was added as a source of calcium and phosphorus for the shells of snail.

Source of the three breeding stock

The giant land snails (*Archachatina marginata*) were from Moor plantation Nigeria, while the garden snail (*Archachatina archachatina*) and the "Long mop" (*Archachatina fulica*) were provided by the South West Forestry officials (from the wild).

Fig 2: The 03 snail Species

Management

Feeds and feeding system

The feeds were sourced for locally. The snails were fed with fresh pawpaw leaves, ripe pawpaw fruits, okra leaves, ripe plantain pills, cassava flour and sweet potato leaves. This was provided about 4-5 times each week for the duration of the trial.

Scoring preference

This was done using a kilogram of each feed item to feed each snail species. The remnant of each feed item (if any) is collected the next morning and weighed. This exercise was done repeatedly each day for the duration of the trial and scoring was done by; total consumption by the snails is scored 100%, 75% consumption was termed highly consumed i.e., 50% was termed moderately consumed i.e. and 25% consumption was considered just a little bit consumed .

Water

Water was provided in water troughs (a rectangular shaped metallic container of about 20cm long and 10cm wide). Water was also used to moisten the soil by sprinkling. This was done each time the snails were fed or when places are hot.

Other management practices

The surrounding was constantly kept clean. Izal (disinfectant) was used to keep away any predators like ants. The cages were kept in an abandoned green house whose roof was reinforced with thatches. Meanwhile[9] reported that the best temperature for normal growth of snail is about 21-23^{0}C.

The incubator

This was a separate cage of each snail species in which the same soil mixture was filled to about 10cm deep. Upon laying, the eggs were collected and taken to the incubator using a trowel. Small holes of

about 2-3cm deep were made on the soil, and the batch of eggs is place in each hole, covered with the soil and a labeled tag attached to each hole. This labeled tag contains the snail species, the number of egg laid in that batch and the date of lay.

Data collection / weight of snails

The initial weights of all the 03 snail species were recorded separately and used as covariance. The exercise was repeated after 24 weeks.

Egg weight

For the separate species and for each snail, the number of eggs in the batch is counted, and the eggs weighed one after the other to the nearest 0.01g.

Incubation period

Each variety had its own incubator. Upon laying, collection of the eggs and weighing, the batch of egg is taken to the respective incubator. The batch of eggs is labeled for the date it was incubated. This was then monitored until the eggs get hatched completely.

Collection and weighing of the hatchlings

After the hatching of each batch of eggs, the hatchlings (or baby snails) were collected, counted and weighed. While the un-hatched eggs are equally noted and recorded.

Hatchability

This is calculated for each batch of egg and snail species by finding the percentage of the number of hatched eggs on the total number of eggs incubated.

Weight of Growers

The weights of each variety of the hatchling were taken after 8 weeks. After 2 months baby snails or hatchlings are called growers. Each grower snail was weighed separately and the average taken for snails of the same batch and species.

Proximate analysis of some of the feed items

The proximate analysis of the feed items was carried out described by AOC[10] method. Experimental design and analysis: Randomized complete design was used.

Treatments

There were 03 species of snails with each of them considered a treatment i.e. *Archachatina marginata*- giant land snail as treatment 1-T_1, *Archachatina archachatina* – garden snail as treatment 2 –T_2 and *Archachatina fulica* – "Long mop" as treatment 3-T_3. Each treatment had four replicates with each replicate having twenty five snails. Each treatment had its own set of cages and caution was taken to make sure they were evenly distributed and equally spaced in the green house. All the animals were fed on the same type of feed.

Statistical analysis

All the data collected for the different parameters were subject to analysis of variance (ANOVA) using the GENSTAT[11] software.

Results

Proximate composition of some diet items

The proximate compositions of the dietary items are as shown in table 1:
All the three snail varieties were fed on the same feed at the same rate each time they were fed.

Table1. Proximate composition of dietary items

Nutrient (%)	Feed item					
	Pawpaw leaf	Banana (ripe)	Cassava flour	Oyster shell	Bone meal	Pawpaw fruit
Dry matter	24.82	08.96	90.00	-	-	8.1
Crude protein	31.66	09.59	2.50	-	-	8.8
Crude fiber	8.25	0.52	3.5	-	-	0.5
Ether Extract	0.90	0.3	0.50	-	-	0.1
Ash	9.41	8.96	10.12	-	-	-
NFE	49.78	80.30	53.56	-	-	82.1
Energy(Kcal)	30.00	82.00	3200	-	-	-
Calcium mg/100	-	-	-	35.00	37.00	15.8
Phosphorus mg/100g	-	-	-	-	15.00	11.6

Feed preference irrespective of the snail variety

Feed items	Performance
Pawpaw leaves	Totally consumed
Pawpaw fruit	Highly consumed
Okra leaves	Totally consumed
Cassava flour	Moderately consumed
Ripe banana	Highly consumed
Ripe plantain peels	Just a little bit consume

Performance indices

The results of the averages of weights of the snails, number of eggs per batch for each variety, weight of hatchlings and weight of growers are presented in table 2.

Table 2: Performance indices for the three edible snail species (Means± SEM)

Parameter	Species			
	T_1	T_2	T_3	SEM
Average weights(g)	491.46[a]	56.40[b]	37.50[c]	6.82
Average number of eggs per batch	8-12	15-25	15-20	-
Average weight of eggs(g)	2.78[a]	0.92[b]	0.96[b]	0.30
Incubation period (days)	25-28	21-23	21-23	-
Hatchability (%)	100	92.86	85.71	-
Average weight of hatchlings(g)	3.35[a]	1.07[b]	1.05[b]	0.39
Average grower weight(g)	220.11[a]	25.40[b]	16.80[c]	5.24
Economic analysis for producing 1 ton @ US$ 50	US$ 350	US$ 500	US$ 250	-

a) SEM = Standard Error of Mean.

b) abc = Means along the same row with different superscripts are significantly different ($P<0.05$)

Average initial weight of adult snails

As shown in Table 2, significant (P<0.05) species effect was observed on the initial weights of the three edible snails. *A. marginata* had the highest initial weight (491.46g), followed by *A. archachatina* (56.40g) and least weight (37.50g) was recorded for *A. fulica.*

Average number of eggs per batch of eggs laid

As shown in Table 2, *A. archachatina* had the highest average number of eggs (15-25), followed by *A. fulica* (15-20).

Average weight of eggs in a batch of each species

There was a significant (P<0.05) species effect on the weight of eggs. A. marginata had the highest average weight of eggs (2.78g) while no significant varietal effect was recorded for the garden snail and *fulica*.

Incubation period

There was no difference in the incubation period (21-23days) garden snail and fulica (21-23days) while the incubation period for the giant land snail was 25-28.

Hatchability

As shown in Table 2, there were slight variations on the hatchability with the giant land snail having a percentage hatchability of 100%, garden snail had 92.86% and fulica had 85.71%. On the whole the percentage hatchability was relatively high compared to other livestock species like poultry, guinea fowl and geese.

Average weight of hatchlings

The results of average weight of hatchlings in Table 2, shows significant (P<0.05) varietal/ species the giant land snail having the highest (3.35g) average hatchling weight, while No significant differences were observed for the garden and fulica snail species as they both had almost the same weight for the hatchlings (baby snail).

Average grower weight

Also significant treatment effect (P<0.05) was recorded for the weight of grower snails. Highest grower weight (220.11g) was recorded for giant land snail, while 25.40g and 16.80g were recorded for the garden and fulica respectively.

Discussion

Performance indices of the three edible snail species

The results obtained in this study indicate that, the three edible snail species can conveniently be housed using intensive cage housing system with a guarantee for optimal performance. Good management practices such as proper feeding; good ventilation, shade cover, good soil and proper stocking density are necessary conditions for optimum performance of snails. This same observation was reported[12,13] in FAO report.

From the average weight of the eggs, growers to adults, of the three species, giant land snails had the highest weight followed by garden snail and least was *fulica*. This difference in average weights was more of genetic rather than the performance influenced by the feed or housing system. Considering the average number of eggs per batch of eggs laid, garden snail had the highest number of eggs per batch (15-25 eggs). This high number of eggs for garden snail can be seen as a compensation for its low weight as the population of snails

generated in a given batch of snail can be compared to the small snail population from giant land snail with a high weight. These results agree with those of Etchu[14].

The results observed in this study reveal a correlation between the average weight of eggs and the incubation period of the eggs of the three species. The incubation temperature varied from 24^{0}C to 25.5^{0}C. The eggs which are spherical and translucent and golden yellow in colour have an incubation period which varied from 21-23 days for the garden to 25-28 days for the giant land snail. In this study and irrespective of the species, the eggs laid by the individual snail do not all hatch at the same time. It appears that some eggs will hatch day(s) earlier while others may take additional 1-2 days for last baby snail to emerge. This same observation was reported[2]. The reason for this variability is probably due to the fact that all the eggs in a given batch are not laid at the same time thus the variation in the hatching period. The results presented on hatchability for the three edible snail species are above the 80% observed[14], on Helix aspira. Irrespective of the species, the eggs are hatched inside the soil where they first feed on the shell before the hatchling move to the surface of the soil.

Unlike in poultry[14], hatchability in snails is not a function of the egg weight. Though with few exceptions, as observed in this study, all the unhatched eggs had lower weights than the hatched once. The baby snails just hatched are fragile and can easily be damaged and destroyed, so care should be taken when removing them from the incubator to the nursery. What was equally observed in this study was that irrespective of the species, the hatchlings consumed their shells as their first food. The size of any snail at any given time is a function and reflection of its species. Irrespective of the method and type of feeding or housing system, no snail can be bigger than the size of the species to which it belongs. The sizes are species specific and each has a way through which economically, it compensates for any deficiency associated to that species. This explains why the giant land snail with its few number of eggs per batch (8-12) has a large body weight, while the small sizes of garden snail is compensated by the many eggs it lays per batch (15-25). As mentioned earlier, this study is one of the pioneer works in this aspect (reproductive physiology) in snails; few literatures were available for citation.

Conclusions

The Three edible snail species giant land snail, garden and fulica with good management practice, can conveniently be housed using intensive cages system with guarantee for optimum performance. Also the short production cycle, coupled with efficient feed utilization makes snails the ideal opportunistic animal for improved nutrition and income generation to farmers both on and off season with additional advantage of combining it with other farming practices.

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SHORT COMMUNICATION

MILK OFFTAKE OF RENDILLE CAMELS IN THE MOBILE AND SETTLEMENT CAMPS IN NORTHERN KENYA.

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Camels are the only livestock kept by pastoral communities that provide milk all the year round[1]. Some pastoralists today consider the camel to be potentially the most important source of food in arid lands especially during drought periods[2]. In terms of food security, the camel is considered to be superior to all other livestock species. Camels have a longer lactation period and yield more milk in comparison to cattle and goats[3, 4].

In Kenya, among the Rendille pastoral community, the percentage of camel milk consumed by pastoral households is close to 100% of the overall milk consumption during dry season and droughts[5]. Some family members, such as herders in the mobile camps obtain nearly all their calorie intake from milk, meat and blood during certain times of the year supplementing their diet with grains only during extreme periods.

Camels feed on a wide range of fodder plants which are avoided by sheep and cattle, such as salt bushes *Sueda monoica*, *Salvadora persica* and also some aromatic species[6], hence their better survival capability. Their diverse feed range declines offset of other species during dry season and contribute to a broad and stable niche[7].

Despite the significant role of camels among societies, approaches toward improving benefit from camel husbandry are still lacking. This is also due to the fact that most studies had little general application to practical aspects of camel production under pastoral conditions[8].

The Rendille camel keepers distinguish the camel types Dabakh, Godan and Coitte according to their milk performance and adaptation[9]. However, studies conducted on milk performance of camels and their distribution in nomadic pastoral areas are few, and most of them have been carried out under confined conditions as shown in the review by Simpkin[10].

The aim of the study was to asses camel keepers' strategy to meet the household needs for milk in settlement and mobile camps.

The study was conducted in Marsabit district in northern Kenya in the two divisions, Loiyangalani and Laisamis, which are areas inhabited by the Rendille camel keepers. Within the divisions, four localities were further identified as specific study sites. The localities were Ngurunit, Namarei, Korr and Kargi situated within a radius of 80 to 150 kilometers from Marsabit town. The study area covers about 11,000 square kilometers between 2^0 and 3o north longitudes to 37^0 and 38^0 east. The study was conducted between the months of March to May 2003.

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Questionnaire interviews were conducted during daytime from 0900 hours to 1600 hours. The following information was recorded on an individual record sheet: herd type, type of camel, camel parity, lactation month, frequency of suckling, number of teats milked, condition of the camels, condition of the calves, access to salty areas and frequency of watering.

The mobile and settlement camps were selected purposively in each location (randomization was not possible because of the nature of the place and the nomadic lifestyle of the camel keepers), interviews were conducted with 172 camel keepers who kept lactating camels. About 10 milk measurements were supposed to be recorded for each type at each lactation month, ranging from first to twelfth lactation month.

The least square means of mean daily milk offtake was analysed for both settlement and mobile camps using analysis of variance according to the General Linear Model Procedure of the SAS[11].

Out of a total of 162 camels that were in settlement camps, 19% were in the first and second lactation month, 67% were at third, fourth and fifth lactation months. Camels in the seventh up to twelfth lactation month only made up for 24% of the camels.

Out of 137 camels found in the mobile camps 63% were in seventh to twelve lactation months, 29% were in third, fourth, fifth and sixth lactation month, while the remaining 8% were in first and second lactation month .

Mean daily milk offtake for camels in settlement camps was significantly higher ($p<0.001$), than for camels in the mobile camps (Table 4). Camel keepers preferred to keep camels at peak lactation months (three and four) closer to the homesteads. Milk offtake of the three camel types was found to be significantly different. A similar type of variation has been reported in Somali camels in eastern Ethiopia[12]. Milk offtake in settlement herds was found to higher than in mobile camps.

This is in agreement with the findings of Njanja and Oba[13] that peak yields occur during the early lactation stages. The camels were alternated across the camps depending on the availability of pasture and status of production, to ensure that milk is available all the year round especially to the young children who are mainly in the settlement camps. This is in agreement with Martha[14] that Rendille children among the nomadic pastoralists suffered low levels of malnutrition during dry seasons compared to children around urban centers mainly because they consume three times as much milk as the sedentary children.

Table 1: Rendille names of the years

Name	Sabdi	Ahdi	Alasmin	Talatha	Arbah	Khamis	Gumadi
Year	1987	1988	1989	1990	1991	1992	1993
	1994	1995	1996	1997	1998	1999	2000
	2001	2002	2003				

Table 2: Rendille names of the months

Month	January	February	March	April	May	June	July
Rendille name	Dibial	Harafa	Daga	Ragar I	Ragar II	Heidi Keikye	H Boran I
Month	August	September	October	November	December		
Rendille name	H Boran II	Sonder I	Sonder II	Som	Furam		

Table 3: Proportion of camels by herd type

	n	Settlement	Mobile
Dabach	115	66.9%	33.1%
Godan	107	44.9%	55.1%
Coitte	77	48.1%	51.9%

Table 4: Mean daily milk offtake in the two different herd types

Herd	Mean daily milk offtake [l]	Standard error
Settlement	1.99^{a}	0.0696
Mobile	1.53^{b}	0.0744
$R^2 = 0.11$		

These results are in disagreement with that obtained by King[15] who reported that around settlement camps forage is scarce and of low energy value, while in mobile camps forage is more abundant and of high energy value but water is scarce. It therefore means that milk production would have be higher in the mobile camps. However, it all depends on the individual camel type, production status and the pastoral management strategies in place.

Although the study found that there were high amounts of milk available in the settlement camps compared to the mobile camps, the camels were frequently interchanged between the camps depending on their status of production and camel keepers demand. Furthermore, pastoralists' strategies are designed to minimize risks of destitution and not to maximize production[2]. The two herd groups have been accessed to forage of different nutrient content and different watering levels among other factors.

As more productive camels are transferred to settlement camps at a certain lactation stage, there is need to sensitize the pastoralists on the methods aimed at improving management practices towards further reduction in the magnitude of milk offtake deficit.

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SHORT COMMUNICATION

BRUCELLOSIS AS A ZOONOSIS IN CHIFRA DISTRICT, AFAR REGIONAL STATE, ETHIOPIA

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Brucellosis is one of the major zoonoses in the world[1]. Human brucellosis is commonly caused by exposure to infected livestock and consumption of livestock products (mostly raw milk and milk products). The clinically most important causative bacteria in humans in pastoral areas are *Brucella melitensis* (small ruminants) and *B. abortus* (cattle)[2].

In animals, brucellosis mainly affects reproduction and fertility, reduces survival of newborns and reduces milk yield though, mortality of adult animals is insignificant[1]. It is generally agreed that the impact of the disease in animals is greater in terms of the adverse effects it may have on human health in rural population[3].

In Ethiopia, brucellosis is endemic and reports from different authors indicated that the disease is highly prevalent in cattle, camels and small ruminants in pastoral areas[4,5]. With regard to human brucellosis in Ethiopia, case reports were documented by Alemayehu[6] on a 12 years old girl, by Seboxa[7] on four patients and a seroprevalence rate of 4.8% was reported by Kassahun *et al.*[8] in occupationally exposed groups in Addis Ababa. So far no comprehensive studies have been carried out on human brucellosis in pastoral areas of Ethiopia although animal husbandry is traditional, habit of drinking raw milk is a deeply established culture and the close contact between human and livestock is very high. Therefore, this study was undertaken to determine the seroprevalence rate of human brucellosis and identity the contributing potential risk factors for the occurrence of the disease in Afar pastoralists.

This cross-sectional study was conducted in Chifra district, Afar Regional State, in east and northeastern part of Ethiopia from December 2005 –June 2006. Most people in the district lead a pastoral way of life by rearing camels, cattle, goats and sheep[9].

Ninety-one human serum samples were collected from patients admitted to Chifra Health center with different health problems. Besides, relevant information regarding risk factors (habit of drinking raw milk, habit of eating raw meat and exposure to infected materials like aborted fetus) and clinical manifestations pertaining to brucellosis in human were also gathered during sera collection by using pre-tested and structured questionnaires. The study subjects were categorized into five groups as less than 15 years, 16-30 years, 31-45 years, and 46-60 years and greater than 61 years to see the age distribution of brucellosis in human according to social status (Table 1). This study was part of an ongoing ethically cleared project by Research and Ethical Clearance Committee (RECC) of the

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Ethiopian Health and Nutrition Research Institute (EHNRI).

Rose Bengal plate Test (RBPT)

Serum samples collected were screened for the presence of *Brucella agglutinins* by the Rose Bengal Plate Test (RBPT) according to Nielsen and Dunkan[10] using the *Brucella abortus*[1] strain S99 as antigen. Sera were screened at the Zoonoses Research Laboratory of the Ethiopian Health and Nutrition Research Institute (EHNRI). Those sera which were found to be positive to the Rose Bengal Plate Test (RBPT) were further subjected to Complement Fixation Test (CFT).

Complement Fixation Test (CFT)

All the reagents required for the CFT were evaluated by titration following the standards given by the OIE[11]. CFT was done at the National Veterinary Institute (NVI), Debre Zeit, and all sera giving 75% fixation of the complement at a dilution of 1:5 and above were taken as positive Dohoo *et, al.*[12]. Data collected both from serological tests and questionnaires survey were systematically entered in Microsoft Excel and transferred to Intercooled Stata 7.0 Software to be analyzed. P values indicating statistical significance were considered when they were less than 0.05.

Table 1: Human brucellosis seropostivity according to age and sex distribution in Chifra District, .Afar, Ethiopia

	N^{o} of serum tested	Positive with CFT*	Prevalence rate (%)
Age groups (year)			
<15	6	3	50
16-30	59	8	13.6
30-45	14	4	28.6
46-60	5	0	0
> 61	7	0	0
Total	91	15	16.5
X^2 (4) = 9.1169, P = 0.058;		*95% CI = [0.2073 – 1.051]*	
Sex			
Male	33	2	6.1
Female	58	13	22.4
Total	91	15	16.5
X^2 (1) = 4.0819, P = 0.043;		*95% CI = [0.4435 – 16.028]*	

CFT* = *Complement Fixation Test*

Table 2: Distribution of human brucellosis seropostivity in relation to selected risk factors at Chifra District, Afar, Ethiopia

Risk factors	Total sample	Positive (%)	(CFT) χ^2	95. % CI	P value
Close contact with animals	63	14 (22.2)	4.898	[0.415-41.9]	0.027
Habit of drinking raw milk	40	11(27.5)	6.037	[1.082-15.95]	0.014
Handing of parturient					
Material	22	8.(36.4)	8.3297	[0.815-12.26]	0.004
Habit of eating raw meat	7	-	-	-	-

Out of 91 human sera tested 15(16.5%) were found to be positive for brucellosis. Among these 13(86.7%) were female and 2(13.3%) were male (Table 1). There was a statistically significant difference between males and females in seropostivity (χ^2 = 4.0859, P <0.05). The difference in seropositivity among the different age groups was not significant (χ^2 =9.1169, P >0.05). However, the highest proportion of sero-positive individuals were observed at age group <15 years (Table 1). Out of the 15 positive individuals 14(93.3%) had history of close contact with animals, 11(73.3%) had habit of drinking raw milk and 8(53.3%) handling of parturient materials. However, none of them had the habit of eating raw meat. There was a statistically significant (P<0.05) associations between the above-mentioned risk factors and seropositivity (Table 2). The most common symptoms mentioned by those positive individuals were; fever 15(100%), weakness 11(73.3%), sweating 6(40%), headache 14(93.3%), joint pain 7(46.7%), back pain 8(53.3%) and chills 13(86.7%). One woman was also found to have had a history of abortion. The questionnaire survey also indicated that none of the respondents had heard of any information about the disease previously.

The results of this study indicated that, the prevalence of human brucellosis in Afar Chifra District is high with an overall prevalence rate of 16.5%, which is consistent with previous work reported, by Taye[13]. Similar observation was also recorded in Saudi Arabia[14]. It is not surprising to get high prevalence of human brucellosis in pastoral communities where people's life is entirely dependent on livestock along with the presence of infected animals. Furthermore, Madkour[15] and Omer *et al.*[16] reported that the prevalence of human brucellosis in a given area is largely influenced by the prevalence of the disease among domestic animals that are found around him.

In the present study, strong association was observed between seropostivity and handling of parturient materials followed by drinking raw milk, which is in agreement with other studies[14,17,18,19]. This study indicated that consumption of raw meat was not considered as possible risk factor for *Brucella* infection in the area, because consumption of raw meat is not a common practice. However, Kassahun *et, al.*[1] reported the risk of contracting Brucella infection by consumption of raw meat especially in urban areas whereby raw meat eating is very common. In contrast to other studies on human *brucellosis*[20,21] females, were found to be more prone to Brucella infection and susceptible to brucellosis than males. In agreement to this study similar report in Saudi Arabia[14] indicated that the exposure rate and the proportion of seropostivity were higher among females than males. This may reflect cultural and social behaviour patterns whereby the females are actively involved in caring for domestic animals in pastoral areas.

In this study, higher proportion of seropostivity was observed in children within the age category (<15 years), although, the different age categories did not differ significantly. In contrast to the finding of this study peak incidence of brucellosis in the age groups >40, 20-45, >25 and 30 to 40 years have been reported by Mussie[21], Hadad and Smith[22], Kassahun *et al.,*[8] and Rana *et al.,*[23], respectively. This difference might have been associated with the fact that in the study area children who are < 15 years of age were responsible to attend small ruminants and had intimate and continuous contact with animals.

The clinical manifestations pertaining to human brucellosis found in this study was

similar to previous findings reported[14,17,21]. Apart from general clinical manifestations, a woman who was found positive by both serological tests also had history of abortion.

The prevalence of brucellosis in human seems to be widespread in Chifra District, Afar Regional State. In agreement with established evidences, this study also indicated that both handling of parturient material and drinking of raw milk as important risk factors for the widespread occurrence of human brucellosis in the area. Milk being the primary food source in pastoral areas like Afar Region, when this is combined with the deeply established taboo associated with the boiling of milk in the culture of these communities, the significance of the disease is considered to be high. Therefore, further study should be conducted to substantiate the above findings so that the actual prevalence of the disease is determined and an acceptable method of prevention of infection with *Brucella* will be implemented. Additionally, there is a strong need to create awareness among the pastoral communities about the nature of the disease and the dangers associated with the drinking of raw milk.

The role of clan leaders who have a strong influence in pastoral communities like the Afar in convincing the nomadic pastoralists against the dangers of contracting brucellosis associated with the habit of raw milk drinking is high and this may serve as an entry point to create awareness to the community about the disease and its pathological effects.

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