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FORMULATION AND *IN VITRO* RELEASE STUDIES ON CHITOSAN-ALGINATE MICROCAPSULES MODIFIED FOR FISH VACCINE DELIVERY

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FORMULATION ET ETUDES *IN VITRO* SUR LES MICROCAPSULES CHITOSAN-ALGINATE MODIFIEES POUR L'ADMINISTRATION DE VACCINS POUR LES POISSONS

Résumé

La présente étude examine la libération *in vitro* de *Vibrio bacterin*, un vaccin pour les poissons produit à partir de microcapsules chitosan-alginate modifiées de HPMCAS pour l'administration par voie orale chez les poissons. Les microcapsules ont été préparées avec la méthode de coacervation counterion en utilisant un générateur électrostatique de gouttelettes. Le noyau alginate de microcapsules a été modifié avec HPMCAS à divers ratios d'alginate (A) : HPMCAS(H) de 1 :1 (AH) ; 1 :2 (AH_2) et 2:1 (A_2H). La libération du vaccin a été étudiée *in vitro* à différents pH du milieu d'élution étalant le pH de l'intestin du poisson, et les données obtenues ont été insérées dans différents types de libération. Les microcapsules d'AH ont produit la plus faible quantité de libération de vaccin de 18,2% après 9h et la quantité était très différente ($p < 0,05$) du témoin. On a trouvé que la libération du vaccin était dans l'ordre suivant : $AH < A_2H < AH_2$ < témoin au pH 1,2. Il n'y avait pas de différence significative ($p > 0,05$) en termes de libération du vaccin entre les microcapsules modifiées et le témoin au pH 3, 6 et 8. Cela était attribué à l'effet entérique de HPMCAS. On a aussi noté que la libération de protéine des microcapsules était contrôlée par la diffusion. La modification de microcapsules de chitosan-alginate est une méthode potentielle pour la préparation de formules de vaccin par voie orale pour les poissons.

Mots-clés : Microcapsules, *Vibrio bacterin*, chitosan-alginate, vibriose, administration de vaccins pour les poissons.

Summary

This study examines the *in vitro* release of *Vibrio bacterin,* a fish vaccine, from HPMCAS modified chitosan-alginate microcapsules designed for oral delivery in fish. The microcapsules were prepared by the counterion coacervation method using an electrostatic droplet generator. The alginate core of the microcapsules was modified with HPMCAS at varying alginate (A): HPMCAS (H) ratios of 1:1 (AH), 1:2 (AH2) and 2:1 (A2H). Vaccine release was studied *in vitro* at different pH of the elution medium spanning the pH of the fish gut, and the data obtained were fitted into different release models. AH microcapsules effected the lowest amount of vaccine release of 18.2% after 9 h and was significantly different ($p < 0.05$) from the control. Vaccine release was found to be

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in the order: AH < A2H < AH2 < control at pH 1.2. There was no significant difference ($p>0.05$) in vaccine release between the modified microcapsules and the control at pH 3, 6 and 8. This was attributed to the enteric effect of HPMCAS. Protein release from the microcapsules was found to be diffusion-controlled. Modification of chitosan-alginate microcapsules is a potential approach for preparing suitable oral fish vaccine formulations.

Key words: Microcapsules, *Vibrio bacterin,* chitosan-alginate, vibriosis, fish vaccine delivery.

Introduction

Vibriosis is a systemic bacterial infection of primarily marine and estuarine fishes, caused by bacteria of the genus 'Vibrio[1]. It is a major cause of mortality in mariculture operations. It sometimes also occurs in freshwater species[2]. During the last 30 years, much interest has been expressed in immunization as a means of preventing vibriosis. Intraperitoneal injection of the vaccine *(Vibrio bacterin)* is probably the most effective method of delivery and produces a high degree of immunity; however, it is expensive, labor-intensive, and stresses the fish[3]. While oral administration would be preferred, therapeutic proteins are poorly bioavailable when administered via the oral route due to acid and enzyme degradation in the stomach as well as poor absorption because of their large molecular size. Recent studies suggest that improved oral protein bioavailability can be achieved by microencapsulating the protein with certain polymers to facilitate delivery to the region of optimal absorption in the large intestine[4, 5, 6]. The fish gut is similar to that of humans in terms of enzyme and acid secretions. In fish stomach, pH range from 1.5 - 5 have been reported[7]. The pH was affected by the presence of food, time of the day the samples were collected for analysis, specie of fish and geographical location. On the other hand, intestinal pH ranges from 6.5 - 7.5[7,8]. However, the transit time of food in the fish gut vary significantly with the size of the fish[9].

Alginate and chitosan are naturally occurring polymers. While alginate is composed of repeat units of a-L-guluronic and ß-D-manuronic acids, chitosan is composed of D-glucosamine residues which are essentially made up of deacetylated chitin. A combination of chitosan and alginate has been reported to form excellent drug delivery device for bioactive agents and living cell immobilization[10] because of the combined effect of the gelling property of the alginate and the mucoadhesive property of chitosan[10, 11, 12]. However, further studies have shown that chitosan-alginate delivery systems are not efficient in protecting proteins from the harsh conditions in the stomach due to the solubility of chitosan in acid pH[5, 6].

Hydroxylpropyl methylcellulose acetate succinate (HPMCAS) is a semi-synthetic pH-sensitive enteric polymer derived from cellulose. It is insoluble in acid pH but soluble at neutral pH and has been used in protecting drugs from acid degradation in the stomach[13]. *In vitro* efforts at delivering proteins orally using microcapsules have been made[5, 6, 14] but no attempt directed at

the formulation of microencapsulated fish vaccine has been reported.

The rationale for this work is that if the dried microcapsules containing the vaccine are incorporated in the pelletised fish feed, then fish immunization can be greatly facilitated. This will be particularly beneficial to young fishes that are more susceptible to high mortality from both disease and harsh vaccination procedures. This study therefore is an exploratory *in vitro* attempt to elucidate the probable influence of selected simulated fish gut pH conditions on the release of a fish vaccine, *Vibrio bacterin* from chitosan-alginate microcapsules modified with the pH-sensitive polymer, HPMCAS.

Materials and Methods

Sodium alginate (Kelco, Chicago, IL) and chitosan (medium viscosity grade) Vansom Chem. Co., Redmond, WA were the polymers used for microcapsule production. *Vibrio bacterin* (used as a 'model' protein) was a gift from Microtek Research and Development Ltd, Victoria, BC, Canada. Hydroxypropyl methylcellulose acetate succinate (HPMCAS) was manufactured by Shin-Etsu Chemical Co. Ltd, Tokyo, and kindly supplied by Biddle Sawyer Corp., New York. Calcium chloride dihydrate, hydrochloric acid and sodium hydroxide were produced by BDH Chemicals, Toronto, Canada. All other chemicals were of reagent grade.

Preparation of encapsulating solution

0.1% of Chitosan solution was prepared by dissolving 1g of chitosan in 900 ml of distilled water containing 10 ml of glacial acetic acid with the aid of a magnetic stirrer. The viscosity (average molecular weight) was reduced to 2.5 x 105 by digesting overnight with 2.14 ml of 1%w/v sodium nitrite solution, i.e., a ratio of 0.05 mol NaNO3 to 1.0 mol chitosan12. 20 g calcium chloride dehydrate and 50 mg Pluronic F68 (a surfactant) were dissolved in the solution and its pH adjusted to 5.55 using NaOH pellets and solution. The solution was filtered and its volume made up to 1000 ml.

Preparation of core materials

1.5 g of sodium alginate was dispersed in 50 ml of distilled water and stirred using a magnetic stirrer until it dissolved. The volume was then adjusted to 100 ml. A 1% hydroxypropyl methylcellulose acetate succinate (HPMCAS) solution was made by first dispersing 1 g of the polymer in 80 ml of 0.017 M sodium hydroxide solution with stirring. Complete solution was then achieved by drop wise addition of a dilute sodium hydroxide solution while stirring. The pH was adjusted to 5.8 with dilute HCl and the volume made up to 100 ml with distilled water. A 0.5 % HPMCAS solution was obtained by diluting the 1% solution 1:1 with distilled water, while for the 5% HPMCAS solutions, 5 g of the polymer powder were dispersed in 0.05 M sodium hydroxide solution, and then dissolution was achieved as described for the 1% solution.

Microencapsulation of vaccine

Sodium alginate solution (10 ml) containing 1.5% *Vibrio bacterin* vaccine alone or in combination with either 1.5, 3, or 6 ml of 5% HPMCAS solution (to produce microcapsules with core alginate:HPMCAS ratios of 2:1, 1:1 and 1:2, respectively,) was extruded into 70 ml of the chitosan solution in a glass petri dish with the aid of an electrostatic droplet generator (Model 30R, Bertan Associates, Inc, USA). In this set-

up, the alginate was extruded through a 22G, flat-tipped stainless steel needle (Chromatographic Specialties, Ontario, Canada) with the aid of a syringe pump (Model 230A, Cole-Parmer Instrument Company, Illinois, USA). As the liquid was forced out of the end of the needle by syringe pump, the droplets were pulled off by the combined action of gravitational and electrostatic forces. The potential difference was set at 25 kV on the voltage power supply system and a maximum current of 0.4 mA. The 'live' cable of the voltage power supply equipment was connected to the needle while the ground cable was placed in the collecting solution. The extrusion rate and distance (i.e., distance between the needle tip and the surface of the collecting solution) were fixed at 10 ml/min and 10 cm, respectively.

On reaction with Ca^{2+} in the chitosan solution, the polyanionic sodium alginate droplets gelled into calcium alginate beads, which rapidly reacted with the polycationic chitosan. The resulting microcapsules were allowed an additional reaction time of 2 min following the termination of alginate extrusion, and then examined by a light microscope (Model LS25, Olimpux, Japan) with a calibrated eye piece for size and shape consistency. The chitosan solution was removed from the petri dish by vacuum aspiration, and the microcapsules were further reacted with 0.5% HPMCAS solution for 2 min in order to form an additional coating or membrane around the microcapsules. The microcapsules were then washed twice with distilled water and rinsed rapidly with isopropyl alcohol to remove as much water as possible before air drying at ambient temperature for 24 h prior to conditioning over calcium sulphate granules (Drierite) in a vacuum dessicator.

Vaccine release determination

Dried microcapsules (50 - 60 mg), accurately weighed, were placed in each of 110 ml wide-mouthed glass bottles in a thermostatted shaker bath (Gallenkamp, England) set at 30 ± 0.5^0C. 50 ml of the elution fluid, i.e., 0.1M HCl (pH 1.2) (consisting of 8.5 ml of 3.6g% conc. HCl and 0.2% w/v NaCl dissolved in distilled water), was put in each of the bottles and capped. The shaker bath was agitated at a speed of 80 rpm and elution of *Vibrio bacterin* from the microcapsules was monitored spectrophotometrically (Spectronic 2000, Bausch and Lomb, Germany) at λ_{max} of 280 nm. The experiment was carried out in quadruplicate. This procedure was repeated using elution fluids of pH 3, 6 and 8 to simulate other regions of the gut. Glycine buffer (pH 3) was prepared by dissolving 2 g NaCl and 11.3 g of glycine in distilled water. The pH was adjusted to 3 using 1M HCl and the volume made up to 1000 ml[5]. Acetate buffer (pH 6) was prepared by dissolving 100 g of ammonium acetate and 4.1 ml of glacial acetic acid in 300 ml of distilled water. The pH was adjusted using ammonia and acetic acid, and distilled water used to make up the volume to 500 ml, while tris-chloride buffer (pH 8) was prepared by dissolving 0.3 g of calcium chloride and 0.97g of tris (hydroxylmethyl) methylamine in water; the pH was adjusted to 8 using HCl and made up to 100 ml with distilled water[15].

Assay of the microcapsules for the initial vaccine content (load) was also carried out spectrophotometrically at 280 nm. Thus 50 mg of the microcapsules was 'citrated' by dispersing it in 20 ml of 0.5M sodium citrate in a scintillation vial and kept

Table 1: r^2 (correlation coefficient) and n (diffusion release exponent) values for different release models at various pH of the release media (The control has alginate but no HPMCAS in the core).

pH	Alginate:HPMCAS		zero order	first order	Higuchi	Millar and Peppas
1.2	Control	r^2	0.5642	0.6425	0.6939	0.7057
		n				0.3623
	1:1	r^2	0.5591	0.6141	0.6808	0.6876
		n				0.3936
	1:2	r^2	0.6796	0.7196	0.7555	0.7558
		n				0.5233
	2:1	r^2	0.7651	0.8498	0.8822	1
		n				1
3	Control	r^2	0.5642	0.6425	0.6939	1
		n				1
	1:1	r^2	0.5591	0.6141	0.6808	1
		n				1
	1:2	r^2	0.6796	0.7196	0.7555	1
		n				1
	2:1	r^2	0.6065	0.9841	0.6994	0.6998
		n				0.4708
6	Control	r^2	0.5642	0.6425	0.6939	0.7057
		n				0.3623
	1:1	r^2	0.5589	0.6149	0.6825	0.6896
		n				0.3936
	1:2	r^2	0.6796	0.7196	0.7555	0.6998
		n				0.4708
	2:1	r^2	0.7651	0.8498	0.8822	0.6998
		n				0.4708
8	Control	r^2	0.5642	0.6425	0.6939	0.7057
		n				0.3623
	1:1	r^2	0.5589	0.6149	0.6825	0.6896
		n				0.3936
	1:2	r^2	0.6796	0.7196	0.7555	0.7558
		n				0.5233
	2:1	r^2	0.7651	0.8498	0.8822	0.6998
		n				0.4708

overnight. (Citration was used to break down the gel structure containing the entrapped protein[12]). The microcapsules, together with the citrate solution, were then transferred to a mortar and crushed with a pestle to effect maximum vaccine release into solution[11].

Release Kinetics

Data obtained from in vitro release studies were fitted to various kinetics models to determine the mechanism of drug release from the microcapsules. The release models used were zero order (F = Kt), first order ($F = 100(1-e^{-Kt})$), Higuchi ($F = K^{t½}$) and Millar and Peppas ($F = kt^n$)[17]. Where F = amount of drug released at time t, and k = release constant for the individual equations. These model equations were entered into the regression library of Sigmaplot 9.0 (Sigma Systat Software Inc., CA, USA). The software contains several built-in equations and graphs and has the capability to fit input data into the built-in curves automatically. The correlation (r^2 values) between the predicted values obtained from the models and experimentally observed results were computed[16, 17].

Results

The wet microcapsules appeared spherical with a diameter in the range; 300 – 500 µm. On drying, the capsules assumed an irregular shape and the diameters shrank

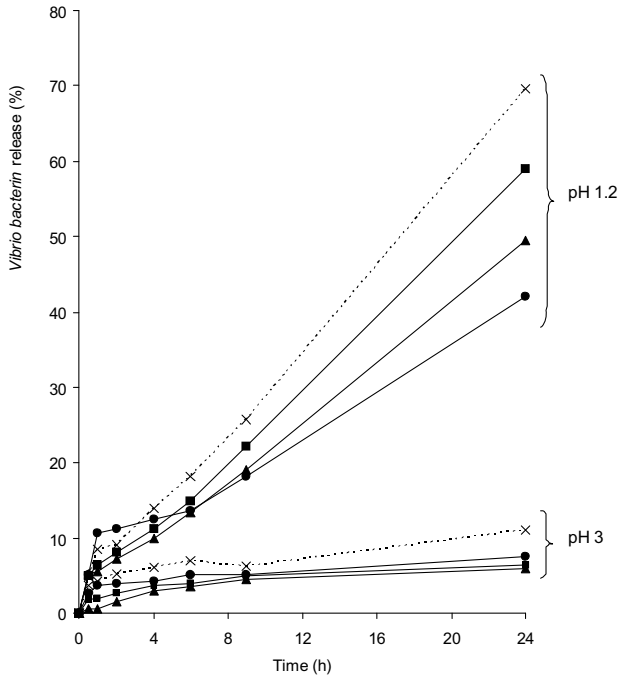

Fig. 1: *Vibrio bacterin* release from microcapsules at simulated gastric pH [alginate:HPMCAS ratio = 1:1 (●), 1:2 (■), 2:1 (▲) and control (x)]

to 100 – 150 µm (approx. one-third of their original value). In the release medium, the dried microcapsules regained their spherical shape.

The effect of the modification of both the microcapsule core and coat with HPMCAS on the release of *Vibrio bacterin* at different pH of the release medium is shown in Figs. 1 - 3. The core of the microcapsules was modified with HPMCAS at the alginate: HPMCAS ratios of 1:1, 1:2 and 2:1 (coded; AH, AH_2, and A_2H, respectively). The control (C) is the unmodified microcapsules, i.e., without any HPMCAS as a coat or in the alginate core. At pH 1.2, which simulates the lower end of fish gastric pH, the AH microcapsules showed the least mean vaccine release of 18 and 42% after 9 and 24 h, respectively, (corresponding to 82 and 58% vaccine retention) (see Fig. 1). This was significantly lower ($P < 0.05$) than the control, which had 25 and 70% vaccine released after the same period. Vaccine release after 9 h from AH_2, and A_2H, microcapsules were, 22.2% and 19%, respectively, and are comparable to AH microcapsules. These values also significantly different ($P < 0.05$) from the control. Thus reduced vaccine release at pH 1.2 was achieved by incorporating HPMCAS in the microcapsule alginate core. When the pH of the release medium was increased to 3 (corresponding to the upper end of gastric pH) (see Fig. 1), vaccine release was not significantly different ($P > 0.05$) from the control as all the microcapsules had less than 12% released after 24 h.

At pH 6 (corresponding to the upper end of intestinal pH) (Fig. 2), over 90% vaccine release from the control was observed after 24 h while microcapsules modified with HPMCAS released approximately 60 - 70%

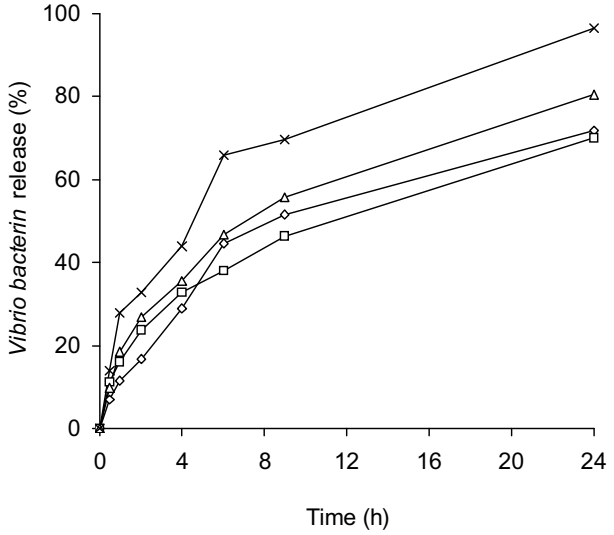

Fig. 2: *Vibrio bacterin* release at pH 6 from microcapsules based on a core alginate: HPMCAS ratio of 1:1 (◇), 1:2 (□), 2:1 (△) and control (x)

of their vaccine over the same period. These values are significantly lower ($p < 0.05$) than for the control. At pH 8 (the highest pH likely to be found in fish intestine), the effect of modification with HPMCAS was not significant ($P > 0.05$) (Fig. 3) except that for the AH_2 microcapsules which showed the lowest vaccine release after 24 h, vaccine release ranged from 65 - 83%.

Discussion

Chitosan constituted the microcapsule membrane while the core was alginate or alginate/HPMCAS. Chitosan, as indicated earlier, is a polycationic polymer which is soluble in acid but insoluble at approx. pH 3 or higher. The microcapsule core in which the vaccine is embedded is a porous gel. HPMCAS is a polyanionic pH-sensitive (enteric) polymer which is insoluble in acid but begins to dissolve as pH approaches neutral[13]. It would appear that an additional membrane or thin film was formed around the chitosan membrane following the reaction of the freshly formed microcapsules with 0.5% HPMCAS solution as described earlier. Thus, at pH 1.2, while the acid-soluble chitosan membrane of the control microcapsules rapidly dissolved thereby exposing the vaccine in the core to leaching, the HPMCAS provided a 'shield' for the chitosan membrane as well as the vaccine by impeding the penetration of the release medium into the core and hence also the outward diffusion of the vaccine from the core.

At pH 3, chitosan membrane retained its integrity in the elution media since at pH 3 and higher, the polymer is insoluble[6]. Thus

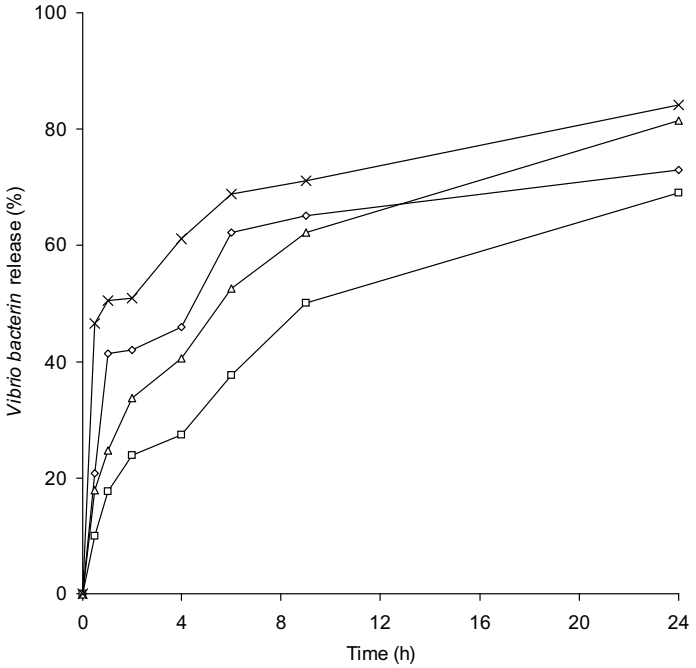

Fig. 3: *Vibrio bacterin* release at pH 8 from microcapsules based on a core alginate:HPMCAS ratio of 1:1 (◇), 1:2 (□), 2:1 (△) and control (x)

vaccine release into the medium might have occurred via the membrane pores. It is likely that the HPMCAS film on the microcapsule membrane and in the core did not substantially affect vaccine diffusion out of the microcapsules. The results obtained at pH 6 and 8 are not significantly different ($P > 0.05$) and hence a change in pH from 6 to 8 did not significantly influence vaccine release from the microcapsules. This is probably due to the fact that HPMCAS, being soluble at ≥ pH 6, is readily dissolved by the elution fluid and, consequently, does not constitute a barrier to vaccine elution as was observed at pH 1.2 and 3, and as Fig. 4 further indicates. Hence, beyond pH 3, vaccine release was neither significantly influenced by pH nor by HPMCAS.

Vaccine release kinetics

The release mechanism of *Vibrio bacterin* from the different microcapsules was determined by comparing their respective correlation coefficients with those of some kinetic models[16]. It would appear that the mechanism of vaccine release from the microcapsules was diffusion-controlled since the values obtained were above 0.5 in all cases. When the correlations between the predicted and experimental models were compared, it was found, to some extent, to fit the theoretic models, mostly in the following order: Millar and Peppas ($F = kt^n$) > Higuchi ($F = kt^{½}$) > first order ($F = 100(1-e^{kt})$ > zero order ($F = kt$). (see Table 1). However, correlation was less than satisfactory except for release at pH 3 which fitted more closely to first order and Millar and Peppas models with correlation coefficient ranging from 0.98 to 1.

Further characterization was carried out using the equation developed by Korsmeyer *et al*[18] $M_t/M_\infty = kt^n$ which is a modification of the model by Millar and Peppas to evaluate the influence of hydration and swelling on drug release rate; where M_t/M_∞ is the fractional amount of drug released, t is time, k is the release rate constant and n is the diffusion release exponent indicative of the drug release mechanism. n usually assume values from 0.5 - 1[18]. For Fickian diffusion from planer systems, for example, n=0.5; for non-Fickian diffusion, n > 0.5; and for case II diffusion or non-Fickian diffusion from

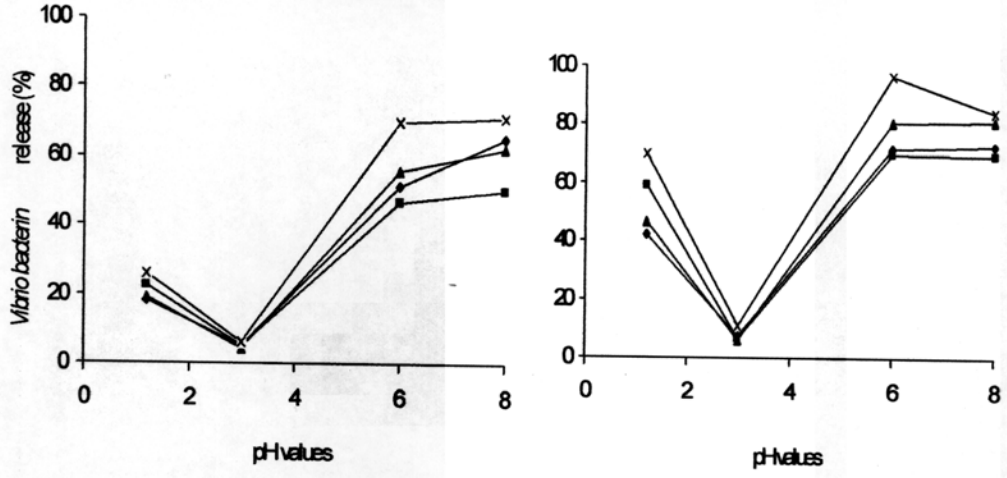

Figure 4: Effect of pH and HPMCAS ratio on *Vibrio bacterin* release after 9 h (a) and 24 h (b) at a core alginate:HPMCAS ratio of 1:1 (♦), 1:2 (■), 2:1 (▲) and Control (x)

spherical systems, n = 1. The values obtained for n were similar to those obtained from the model of Millar and Peppas (see Table 1). Most of the n values, being less than 0.5, suggest that vaccine diffusion was from a combination of factors: swelling, polymeric matrix effect and water filled pores[19] and hence, could not be completely described by known mechanisms. Values of n = 1 suggest non-Fickian diffusion from the microcapsules.

Implication for fish vaccine delivery

As stated earlier, delivery of oral proteins and peptides can only be feasible if they are adequately protected from the hostile acid and enzymes in the stomach to enable them reach that part of the intestine where they should be maximally released and absorbed. While the issue of adequate vaccine absorption at the target region of the gut was not addressed in this work, it seems, however, that by incorporating HPMCAS in the chitosan-alginate microcapsules, high vaccine retention (up to 82%) in the microcapsules is attainable after a 9 h transit from pH 1.2 through pH 3 (where less than 7% vaccine was lost) and then to the intestine where most of the encapsulated vaccine would be released at pH 6 to 8. Extrapolated *in vivo*, it means that this system could facilitate passage of a significant amount of vaccine (>75%) into the colon, which is the region for optimal absorption of proteins and peptides when administered orally. Thus, the results obtained suggest that suitable blending of chitosan-alginate microcapsule core, and coating with HPMCAS may afford a simple and effective approach to protecting encapsulated fish vaccines as they transit through the gastric and upper intestinal regions. These findings are significant with regard to the efforts to develop suitable oral delivery systems for fish vaccines.

Conclusion

This work has shown that modification of chitosan-alginate microcapsules with HPMCAS provides a possible approach for producing microcapsules with reduced vaccine loss in gastric pH conditions. The release kinetic analysis reveal that *Vibrio bacterin* released from the modified microcapsules followed the Millar and Peppas model, an indication that release was diffusion-controlled. Although it appears that blending alginate with HPMCAS in the core as well as the coat around chitosan-alginate microcapsules affords a potentially simple and effective approach to protecting encapsulated vaccine as they transit through the gastric and upper intestinal regions, it would be necessary to test this system *in vivo* using 'live' fish.

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ON – FARM INVESTIATION TO DETERMINE THE APPROPRIATE AGE TO VACCINATE CHICKS WITH THE NIGERIAN INFECTIOUS BURSAL DISEASE VACCINE (FIBROGUMBOVAC®)

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ENQUETE AU NIVEAU DE LA FERME POUR DETERMINER L'AGE APPROPRIE POUR LA VACCINATION DES POUSSINS AVEC LE VACCIN NIGERIAN CONTRE LA MALADIE INFECTIEUSE DE LA BOURSE (FIBROGUMBOVAC®)

Résumé

Nous avons effectué une expérience au niveau de la ferme en vue de déterminer l'âge approprié pour vacciner les poussins avec un vaccin local contre la maladie infectieuse de la bourse -vaccin IBD-(fibrogumbovac®) dans le cadre des efforts déployés pour s'attaquer au problème des échecs des vaccins contre la maladie infectieuse de la bourse.

On a réparti des jeunes coqs âgés de quatre-vingt dix (90) jours en trois groupes : A, B et C ; en subdivisant le groupe C en C_1 et C_2. Les oiseaux du groupe A ont été vaccinés avec le vaccin fibrogumbovac® IBD à l'âge de 10 et 18 jours, tandis que ceux du groupe B ont été vaccinés avec ce vaccin à l'âge de 14 et 35 jours. Les oiseaux du groupe C_1 et C_2 n'ont reçu aucun vaccin IBD. Tous les oiseaux des groupes à l'exception du sous-groupe C_2 ont été soumis à l'infection après la vaccination avec 20% (w/v) de suspension de la bourse de Fabricius confirmée comme étant naturellement infectée par le virus de l'IBD.

Tous les oiseaux vaccinés ont eu une séroconversion avec le Test de précipitation en gélose (AGPT). Le résultat indiquait également que l'administration du vaccin IBD à l'âge de 10 et 18 jours conférait une meilleure protection contre l'IBD à la lumière de la différence observée en ce qui concerne la morbidité et la mortalité entre les oiseaux vaccinés et les oiseaux non vaccinés soumis à l'infection de l'IBD. Toutefois, il n'y avait pas de différence significative d'efficacité (P>0,05) entre les deux modes d'administration de vaccin.

Mots-clés : Age du poulet, vaccination avec le fibrogumbovac®.

Summary

We conducted an on-farm experiment to determine the appropriate age to vaccinate chicks with locally manufactured infectious bursal disease vaccine (fibrogumbovac®) as part of our efforts to address the problem of vaccine failures to Gumboro disease Ninety (90) day old cockerels were divided into three groups: A, B and C with group C subdivided into C_1 and C_2. Birds in group A were vaccinated with the fibrogumbovac® IBD vaccine at 10^{th} and 18^{th} day of age while birds in group B were vaccinated with the vaccine at 14^{th} and 35th day old. Birds in group C_1 and C_2 were not given any IBD

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vaccine. All the birds in the groups except subgroup C_2 were challenged post vaccination with 20% (w/v) suspension of bursa of Fabrius confirmed to be naturally infected with IBD virus.

All the vaccinated birds sero-converted using the agar gel precipitating test (AGPT). The result also indicated that administration of the IBD vaccine at 10th and 18th day of life gave a better protection against IBD based on the observed difference in the morbidity and mortality between the vaccinated and unvaccinated IBD challenged birds. However there was no significant efficacy differences (P>0.05) between the two regimes of vaccine administration.

Keywords: Chicken age, fibrogumbac(R) vaccination.

Introduction

Infectious bursal disease (IBD) first described by Cosgrove[1], is of economic importance in the poultry in Nigeria and other parts of the world. Apart from good husbandry and isolation, the use of vaccine has been widely practised as the method of control of the disease.

These vaccines are referred to as mild, intermediate and 'hot' depending on their effect on the bursa of fabrius[2]. Mild vaccines do not cause bursal damage while intermediate and hot vaccines do[2]. In Nigeria the fibrogumbovac® IBD vaccine produced by the National Veterinary Research Institute (NVRI), Vom, constitutes the bulk of vaccines used for protecting birds against IBD. This Nigerian IBD vaccine is of chicken embryo fibroblast origin and contains a minimum of about 104-5 tissue culture infective dose (TCID50) per bird. Although this Nigerian IBD vaccine has been shown to be safe and able to induce immune responses, the age of birds at vaccination is important[3]. IBD vaccination schedules depend on the maternally derived antibodies in the chicks. Over the years various formulae have been developed to predict the right vaccination age, but some of the variables needed to be taken into account in applying the formulae cannot be readily determined in practise particularly among the local farmers. Therefore, there is a need to conduct field trials with local vaccine in the usual environments in which birds are kept in order to determine the appropriate age for the IBD vaccination.

In this study, we compared two existing field schedules of IBD vaccine administration to chicks with a view to determine which gives a better protection to the vaccinated flock.

Materials and Methods

Site of the experiment

The birds used for this experiment were reared, vaccinated challenged and observed on a small holder poultry unit at Oru-ijebu.

Experimental birds and design

Ninety (90) cockerels of one day old supplied by Eriku Farms Limited, Ijebu-Igbo were used for this study. The birds were kept under an intensive system of management on a small holder poultry unit at Oru-Ijebu. The birds were fed commercial feed also supplied by Eriku Farms Ltd and were given glucose electrolyte in water for the first 3 days. The birds were vaccinated with

Newcastle disease vaccine (NDV) intraocularly when they were 5 days of age. No specific instructions on the management of birds were given to the farmer under whom the birds were kept except to maintain hygiene. However, scientific research protocols were observed throughout the experimental period.

The birds were randomly divided into three main groups: A, B and C with 30 birds per group. Groups A and B represent birds that were IBD vaccinated while group C represents IBD unvaccinated control. Group C was further divided into two subgroups: C_1- challenged, and C_2 – unchallenged containing 15 birds per subgroup.

Serology

Chicks in all the groups were bled by jugular venipuncture at 2nd, 4th and 6th day of age (i.e. before vaccination) and at 42 days of age (i.e. post vaccination). The coagulated blood was left overnight at room temperature to separate the serum. The sera were stored at 4^0C until tested. The immune status of the chicks was determined by use of Agar Gel precipitating test (AGPT) as previously described by Kembi *et al.,*[4].

Vaccine and Vaccination

Four vials of fibrogumbovac® vaccine (NVRI, Vom, Nigeria) were purchased from the Veterinary centre, Ogun State Ministry of Agriculture and Natural Resources, Ijebu-Igbo were used for the study. The vaccines were reconstituted, handled and administered orally according to the recommendation of the manufacturer. Birds were vaccinated twice at 10th and 18th day for group A and 14th and 35th day for group B of life while birds in group C were not given the IBD vaccine.

IBD virus challenge

A 20% suspension (w/v) of bursa of Fabrius harvested from an IBD infected bird was used as challenge virus. The suspension fluid was prepared as previously described by Kembi *et al.*[4] and confirmed for IBD virus with a known positive serum[5]. At 42 days of age, all the IBD vaccinated birds and those in group C_1 were challenged with the IBD

Table 1: Post challenge clinical signs and gross pathology in relations to the regime of IBD vaccination

Clinical Signs	Group A	Group B	Sub group C_1	Sub group C_2
Loss of appetite	2/30	10/30	14/15	0/15
Ruffled feathers	2/30	10/30	14/15	0/15
Incordinaton	2/30	10/30	14/15	0/15
Whitish diarrhoea	2/30	10/30	14/15	0/15
Postration	2/30	10/30	14/15	0/15

Table 2: Post challenge morbidity, mortality and protection in IBD vaccinated and unvaccinated chicks.

Group	No. of birds	Age at vaccination				Post Challenge Morbidity	Mortality	Protection
		10	14	18	35	No (%)	No (%)	No (%)
A	30	+	-	+	-	2(6.7)	2(6.7)	28(93.3)[b]
B	30	-	+	-	+	10(33.3)	6(20)	20(66.7)[b]
C_1	15	-	-	-	-	14(93.3)	12(80)	01(6.67)[a]
C_2	15	-	-	-	-	0(0)	0(0)	15(100)
Total	90							

Figures bearing different superscript within the column differ significantly ($p< 0.05$).

Table 3: Course of experimental IBD in vaccination and unvaccinated chicks.

Morbidity and Mortality	Age	42	43	44	45	46	47	48	49	50	51	52	53	54	55
	days	PC	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Group	A							(2)		2						
	B							(10)		2	0	3	1			
	C_1							(14)	1	3	4	2	1	1		
	C_2							(0)	0	0	0	0	0	0		

PC: Post Challenge
Number in parenthesis indicates morbidity

virus suspension. Each bird received 3 drops (75uL) of the IBD virus suspension:2 drops orally and 1drop intraocularly.

Clinical observation and pathology

Morbidity was determine by counting the number of birds showing clinical signs such as ruffled feathers, incoordination, loss of appetite whitish diarrhoea and prostration. All dead birds were necroptised.

Statistical analysis

The data were subjected to the chi-squared text using vaccine protection of birds and regimes as factors while sample frequencies and percentages were used for other relevant observations[6].

Results

IBD antibodies were detected on the 2nd and 4th and not 6th day of life in all the groups before vaccination with fibrogumbovac®. However, IBD antibodies were detected in all the experimental birds post vaccination. Table 1 shows post challenge clinical signs in relation to the schedule of IBD vaccination that was adopted. Table 2 shows morbidity, mortality and protection post vaccination challenge while Table 3 shows the course of the experimental IBD in vaccinated and unvaccinated chicks. Although vaccination was significantly effective ($p<0.05$) in protecting birds, there was no significant ($p>0.05$) efficacy difference between the two regimes that were evaluated. At post mortem, the carcasses from the groups A, B and C_1 were dehydrated with pinpoint haemorrhages on the thigh and pectoral muscles. The bursa of Fabricius of the group C_1 was swollen with pin point haemorrhages on their proventiculus.

Discussion

There was a history of IBD vaccine administration to the parent stock of the birds used for this study suggesting presence of maternally derived antibodies. However, we could not determine the exact duration and quantity of the materials antibodies in the chicks because of the limitation of the sensitivity of AGPT[2].

The vaccination of each group of birds in this study was done two times (Table 2) because the founding concept of immunization against IBD is constant protection. The detection of IBD antibodies up to 4 days of age suggests presence of maternally derived IBD antibodies. However, the non-detection of maternally derived antibodies in the chicks from the 6th day of age could be due to the inability of AGPT to detect low IBD antibodies[2]. At hatch, birds are usually protected from the disease by maternally derived antibodies transmitted from hen to chicks. The chicks loose these protective antibodies as they grow and become susceptible. Using ELISA technique, it has been reported that maternal antibody titres decreased as the birds grow from 1 to 12 days of age[7].

The clinical signs of IBD were observed among the challenged birds four days post infection (Table 1). This observation supports the three to four days incubation period of IBD[8]. The overall picture of the mortalities recorded in this study is that 2 birds died from group A, 6 from group B and 12 from the unvaccinated group (Table 2) after challenge with IBD virus. These results suggest that vaccination at 10 and 18 days of life provided better protection to birds at risk than the other regime. Although the two regime of IBD vaccination evaluated in this study are consistent with NVRI guidelines, the argument in favour of vaccinating birds at 14 days of age was that interference by the maternally derived antibodies in the vaccinated birds is absent or reduced[9]. However, it was reported by Conte and Borne[7] that a single IBD vaccination in the presence of low maternally derived antibody provided adequate protection to birds.

The course of the disease following IBD challenge in this study lasted 3 days in group A, 6 days in group B and 7 days in the subgroup C_1 even though the incubation period was the same (3 days) in all the infected groups (Table 3). Although vaccination provided significant protection to the birds ($p<0.05$) against IBD, regimes A and B were not significantly different in this respect. However, least numbers of morbidity

and mortality rates were recorded in birds that were vaccinated at 10th and 18th day than those vaccinated at 14th and 35th day of life (Table 2). These results suggest that birds would be better protected when vaccinated with the fibrogumbovac® at 10th and 18th day of life.

The detection of IBD antibodies only in the vaccinated birds indicates seroconversion. However, determining the exact antibody level using ELISA could have explained the efficacy of the vaccine and vaccine regime better. Previous reports from researchers showed variation in the period taken by birds to seroconvert after primary vaccination with the NVRI (IBD) vaccine. For example seroconversion was reported after 14 days[10] and 21 days[4] after primary vaccination, while Ezeokoli *et al*[9] were unable to detect any IBD antibody 6 weeks after primary vaccination. With the present finding the use of immunoprophylaxis programme in breeder hens with fibrogumbovac® is recommended to achieve uniformity in the maternal antibody titre to be transferred to the chicks.The presence of unvaccinated flock in an area where IBD exists may create a break in the immunity pattern of the whole area.

Conclusion

Considering the high susceptibility of chicks at 2-6 weeks of age to IBD virus, the rate of maternal antibody depletion and the time it takes vaccinated birds to seroconvert, we recommend, from our results, that chicks be vaccinated at 10th and 18th day of life when using the NVRI fibrogumbovac® vaccine. We carried out this investigation on-farm to allow farmers participation so that the results obtained are much likely to be transferable into useful information to them.

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AN EVALUATION OF NON-SURGICAL CASTRATION BY SINGLE INTRATESTICULAR INJECTION OF LACTIC ACID IN ADULT MUBENDE GOATS *(CAPRA HIRCUS)*

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UNE EVALUATION DE LA CASTRATION NON-CHIRURGICALE AVEC UNE SEULE INJECTION INTRATESTICULAIRE D'ACIDE LACTIQUE CHEZ LES CHEVRES MUBENDE *(CAPRA HIRCUS)*

Résumé

On a utilisé quarante chèvres (5 groupes de 8) pour évaluer les effets de l'injection intratesticulaire d'acide lactique pour la castration. Le 1er groupe (groupe-témoin) a reçu une injection intratesticulaire d'eau isotonique, tandis que le 2ème groupe était castré à l'aide de la chirurgie. Les 3è, 4è et 5è groupes ont reçu chacun une seule injection bilatérale de 88% d'acide lactique à raison de 1, 2 et 3 ml/10kg de poids vif, respectivement. Les chèvres ont fait l'objet de contrôle quotidien pour observer les réactions de stress telles que les changements d'appétit, la démarche et le comportement. Le sang était prélevé chaque semaine pour l'hématologie, le test de cortisol dans le plasma et le prolan A (FSH). On a également effectué une évaluation du sperme et des tests d'enzymes de tissu testiculaire: la glutamyl-transptidase (GTP) et la déshydrogénase de l'acide lactique (LDH).

Les traitements par la chirurgie et l'acide lactique ont considérablement augmenté ($p<0,05$) le nombre de leucocytes, les taux de cortisol dans le plasma et les taux de FSH, et tous les animaux traités avec l'acide lactique à raison de $\geq$2 ml/10 kg étaient azoospermiques. Les activités testiculaires de GTP et de LDH ont beaucoup baissé à la suite des traitements à l'acide lactique. Nous concluons que les injections intratesticulaires de 88% d'acide lactique à des doses de 2 ml/10kg de poids vif sont efficaces pour castrer les chèvres adultes. Toutefois, les réactions de stress aux injections d'acide lactique étaient presque deux fois plus longues en termes de durée par rapport à la chirurgie, même si les intensités de stress n'étaient pas différentes.

Mots-clés : Acide lactique, castration non-chirurgicale, efficacité, stress, chèvres.

Summary

Forty goats (5 groups of 8) were used to evaluate effects of intratesticular injection of lactic acid for castration. The 1st group (control) received intratesticular injection of saline solution while the 2nd group was castrated by surgery. The 3rd, 4th and 5th groups received bilateral single intratesticular injections of 88% lactic acid at 1, 2 and 3 ml/10kg body weight respectively. Goats were monitored daily for behavioural stress responses such as changes in appetite, gait and demeanour. Blood was sampled weekly for haematology and assay of plasma cortisol and follicle stimulating hormone

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(FSH). Also semen evaluation and assay of testicular tissue enzymes: *Glutamyl-transptidase* (GTP) and Lactate dehydrogenase (LDH) were done.

Surgery and lactic acid treatments significantly ($p < 0.05$) elevated leukocyte counts, plasma cortisol and FSH levels and all animals treated with lactic acid at 2 ml/10 kg were azoospermic. Testicular activities of GTP and LDH significantly declined following lactic acid treatments. We conclude that, intratesticular injections of 88% lactic acid at doses 2 ml/10kg body weight/testis are effective for castrating adult goats. The stress responses to lactic acid injections were however, nearly twice longer in duration compared to surgery though intensities of stress were not different.

Keywords: Lactic acid, non-surgical castration, effectiveness, stress, goats

Introduction

Castration of male animals is a common practice in various livestock production systems[1,2]. Techniques used most widely for castration are: surgery, the clamp (Burdizzo) and the rubber ring (elastrator) methods[3]. There are, however, welfare and cost implications associated with these methods[4,5]. They cause stress to animals and often lead to post-operative complications[4]. The need for pain relief through anesthesia and the treatment required in cases of post-operative complications have associated costs[6]. Furthermore, small-scale farmers can hardly afford to buy the Burdizzo or the ring elastrator[7]. Application of these methods, especially surgery, also requires trained personnel who are unfortunately, not often attracted to rural communities where the majority of livestock are kept[4,5].

Owing to the welfare concerns and prohibitive costs of conventional castration methods, researchers are targeting the development of simple non-surgical alternatives, particularly the use of chemical agents[8,9,10]. Lactic acid is one such chemicals that has been employed for castrating bull calves especially in north America[6,11]. Although lactic acid is cheap and readily available, there is no indication of its adoption for castration in developing countries and apparently, its effects have not been studied in goats[7]. There is also a lingering debate about pain and complications attributable to intratesticular injection of irritant chemicals[11]. This study was hence conducted to evaluate the effectiveness and stress related responses to an intratesticular injection of lactic acid in comparison to surgical castration in adult goats.

Materials and methods

Experimental animals and treatments

The study involved 40 goats aged 6-12 months old and weighing between 13-21kg. The goats were grazed on natural pasture supplemented with crop residues, leafy branches and maize bran containing mineral supplements powder (Twiga Lick powder®, Twiga Chemicals, Nairobi). They were provided fresh drinking water *ad libitum*.

Before commencement of experiments, each animal was clinically certified healthy by thorough physical and laboratory screening. They were then subjected to anthelminthic prophylaxis using moxidectin (CYDECTIN®, Fort Dodge, Spain) at rate of 0.2mg/kg body weight. Animals were allowed

a further two weeks to acclimatise to the station and routine handling. The goats were then divided into 5 groups of 8 animals each. Each goat in the control group received bilateral single intratesticular injection of 2 ml of sterile physiological saline (0.9 % w/v, NS Sodium Chloride Infusion B. P., Marck Parenterals Ltd, India). The second group was castrated by surgery. The remaining 3 groups received an intratesticular injection of 88% lactic acid (BDH Chemicals Ltd, England) at rates 1, 2 and 3 ml/10kg body weight/testis to each goat in groups 3, 4 and 5 respectively. Injections were performed in accordance with Jana et al. (2005)[8].

Clinical examination and collection of blood from experimental animals

Each animal was examined daily for 2 weeks to identify behavioral indices of stress such as abnormalities of posture, demeanor and appetite. Body condition scores (BCS) on a scale of 1-4 and weights of the animals were measured fortnightly for the duration of the experiments. To estimate immediate cellular and hormonal responses to treatments, 4 ml of blood was collected from each goat, immediately after treatment, into heparin-impregnated vacutainers through a jugular venipunture. Blood collection was subsequently repeated every 7 days. To avoid circadian variations in hematological

Table 1: Percentage distribution of goats that showed reduced appetite, abnormal gait and dullness after treatments

Treatment	Number of days since treatment								Total (%)
	D1	D2	D4	D6	D8	D10	D12	D14	
Percentage of goats that showed reduced appetite (n=40)									
Control (saline)	4	0	0	0	4	0	0	0	**8**
Surgery	10	10	6	4	0	4	0	4	**38**
1 ml/10kg	14	10	6	0	0	4	4	0	**38**
2 ml/10kg	16	10	6	4	0	0	4	0	**40**
3 ml/10kg	20*	14	4	6	0	0	4	0	**48**
Total (%)	**54**	**44**	**22**	**14**	**4**	**8**	**12**	**4**	
Percentage of goats that showed abnormality in gaits (n = 40)									
Control (saline)	4	0	4	0	0	0	4	0	**12**
Surgery	6	20*	10	6	4	0	4	0	**50**
1 ml/10kg	20*	14	10	6	0	0	0	0	**50**
2 ml/10kg	20*	16	14	4	4	0	0	0	**58**
3 ml/10kg	20*	20*	16	6	6	0	0	0	**68**
Total (%)	**80**	**70**	**54**	**22**	**14**	**0**	**8**	**0**	
Percentage of goats that showed dullness (n = 40)									
Control (saline)	0	0	0	4	0	0	0	4	**8**
Surgery	14	10	4	0	0	6	4	4	**56**
1 ml/10kg	16	10	4	0	4	0	4	0	**38**
2 ml/10kg	20*	16	10	8	4	0	0	4	**60**
3 ml/10kg	20*	16	14	4	4	4	0	0	**62**
Total (%)	**70**	**52**	**32**	**12**	**12**	**10**	**8**	**12**	

*D = Day, *All (100%) were manifesting reduced appetite, abnormal gaits and dullness. For doses of lactic acid 2 & 3 ml/10kg, percentages of goats with reduced appetite, abnormal gaits or dullness were higher than in surgery group*

Table 2: Effect of treatments within the 1st 8 weeks after treatment

Treatment	Weight gain (Kg)	SE	95% Confidence Interval		p-value
			Lower bound	Upper bound	
Saline (Control)	3.40	0.11	2.95	4.30	0.150
Surgery	3.10	0.09	2.67	4.05	0.210
Lactic acid 1 ml/10kg/testis	3.40	0.08	3.10	4.35	0.120
Lactic acid 2 ml/10kg/testis	3.00	0.08	2.57	3.87	0.360
Lactic acid 3 ml/10kg/testis	2.30	0.10	1.95	3.23	0.027*

** Denoting significant difference ($p < 0.05$) relative to control. Data were estimated marginal means, SE = Standard error*

indices, the samples were collected between 8.00 – 9.00am. About 2 ml of each sample was immediately subjected to hematological determination of packed cell volume (PCV), total protein (TP), total leukocyte and neutrophil counts. The remaining volume was centrifuged and resulting plasma frozen at -20^{0}C for hormonal assays later.

Assay of plasma cortisol and follicle stimulating hormone (FSH) concentrations Quantitative determination of cortisol in plasma was done using competitive ELISA (GmbH, Wiesbaden, Germany). Plasma cortisol concentrations were interpolated from standard curves generated using serum calibrators of cortisol concentrations 0, 10, 20, 30, 60 and 100 ng/ml, measured at 450nm absorbance. For quantitative determination of FSH, an automated Enzyme Linked Fluorescent Assay (ELFA) technique was employed (VIDAS®, BIOMERIEUX®, Lyon, France) and results reported in mIU/ml.

Semen collection and evaluation

Using a ram electro-ejaculator (Medata Systems Ltd., United Kingdom), semen was collected from each goat a week prior to treatment and then 28 days after. The samples were evaluated by gross and microscopic examination[12].

Assay of testicular tissue enzymes

Testes were surgically recovered from 2 animals in each group at 2 and 8 weeks after treatments. Extraction of tissue enzymes was done in accordance with Jana et al (2005)[8]. Glutamyl transpeptidase (GTP) and lactate dehydogenase (LDH) activities were determined by spectrophotometry[13].

Data analysis

Descriptive statistics were performed using the frequency procedure of SAS (Statistics Analysis Systems Institute, Cary, NC). Treatment effects between groups and the time-treatment effects were compared using restricted maximum likelihood (REML) analysis by the SAS mixed model procedure (PROC MIXED)[14]. P-values < 0.05 were considered significant.

Results

Effect of treatments on appetite, gait and demeanor

Compared to control goats (Saline group), percentage distribution of goats that showed reduced appetite, abnormality in gait and dullness was higher in both surgery and lactic acid treated groups (Table 1). Within the lactic acid treated goats, this percentage increased as the dose of lactic acid increased.

Table 3: Parameter estimates for leukocyte counts in goats after treatments

Treatment	Effect	Estimate	SE	t- Value	P-value
	Intercept	5.797	0.4818	12.03	<0.0001
Surgery	Treatment	12.923	0.6813	18.97	<0.0001
1ml/10kg	Treatment	13.172	0.6813	19.33	<0.0001
2 ml/10kg	Treatment	13.266	0.6813	19.47	<0.0001
3 ml/10kg	Treatment	16.129	0.6813	23.69	<0.0001
	Time	0.080	0.0306	0.03	0.010
Surgery	Time-treatment	-0.378	0.0432	-8.72	<0.0001
1ml/10kg	Time-treatment	-0.423	0.0432	-9.78	<0.0001
2 ml/10kg	Time-treatment	-0.536	0.0432	-12.39	<0.0001
3 ml/10kg	Time-treatment	-0.537	0.0432	-12.57	<0.0001

The estimates are REML. All treatments led to significant ($p<0.05$) elevations in leukocyte counts, SE = Standard error

Effect of treatments on body weight

Surgery and lactic acid treatments did not significantly affect weight gain of treated animals compared to the control group except the 3ml/10kg lactic acid treatment (Table 2). Weight gain decreased with increasing dose of lactic acid ($r = - 0.993$).

Effect of treatments on haematological values

Mean PCV values were not affected in all experimental groups. Surgery and lactic acid treatments significantly (REML > ±5.80; $p < 0.05$) increased leukocyte counts (Table 3). There was positive correlation ($r = 0.88$) between leukocyte counts and dose of lactic acid.

Plasma hormone changes

The surgery and lactic acid treatment groups had significantly ($p <0.05$) increased plasma cortisol values except the 1ml/10kg treatment group (Table 4). Unlike in the surgery group (REML =- 0.010; $p > 0.05$), the time treatment effect was significant in the lactic acid (REML =-0.027; $p < 0.05$) treated goats. Compared to the control (saline) group, there was significant ($p<0.05$) elevation in plasma FSH in surgery and all lactic acid treated animals (Table 5). Plasma FSH concentrations increased with increasing dose of lactic acid ($r = 1.00$).

Changes in semen characteristics

Changes in seminal characteristics are shown in Table 6. There was marked diminution in seminal volume for all doses of lactic acid. Ninety-three (93%) of semen samples from lactic acid treated goats were aqueous 28 days after treatment while 7% of samples from the 1ml/10kg treatment group were milky in appearance. Poor motility was observed in 11% of animals from the 1ml/10kg treatment group, which also exhibited *oligospermia*. The 2-3 ml treatments groups exhibited absolute *azoospermia*.

Table 4: Effect of treatments on plasma cortisol concentrations

Treatment	Effect	Estimate	SE	t-Value	p-value
Intercept		29.913	1.4173	21.11	<0.0001
Surgery	Treatment	4.0534	2.0044	2.02	0.0470
1 ml/10kg	Treatment	3.6509	2.0044	1.82	0.0729
2 ml/10kg	Treatment	4.6947	2.0044	2.34	0.0221
3 ml/10kg	Treatment	6.1642	2.0044	3.08	0.0030
	Time	-0.3302	0.0801	-4.12	0.0001
Surgery	Time-treatment	-0.0056	0.1133	-0.05	0.9608*
Lactic acid 1 ml/10kg	Time-treatment	-0.2718	0.1133	-2.40	0.0191
Lactic acid 2 ml/10kg	Time-treatment	-0.2825	0.1133	-2.49	0.0150
Lactic acid 3 ml/10kg	Time-treatment	-0.3505	0.1133	-3.09	0.0029

*In the surgery group, Plasma cortisol did not significantly (p>0.05) vary for the duration of the experiments unlike the lactic acid treated goats.Data presented are REML; Time in days;SE = Standard error

Table 5: Changes in plasma FSH concentrations after lactic acid treatments

Treatment	Plasma FSH Concentration (mIU/ml)				
	Pre-treatment	Week 1	Week 4	Week 8	Week 12
Saline (control)	0.87	0.97*	0.89*	0.91*	0.87*
Surgery	0.89	1.70	3.00	3.3	3.4
Lactic acid 1 ml/10kg	0.92	0.97*	1.27	1.51	1.52
Lactic acid 2 ml/10kg	0.87	1.20	1.36	1.75	1.84
Lactic acid 3 ml/10kg	0.89	1.31	1.48	2.64	2.78

*Not significantly (p > 0.05) different from pre-treatment values. Plasma FSH concentrations tended to increase with time after treatment, IU = International unit

Changes in activities of GTP and LDH

There was significant (p<0.05) diminution in activities of both GTP and LDH following intratesticular injections (Table 7). Reductions in activities of both GTP (r = 0.981) and LDH (r = 0.987) were positively correlated to lactic acid dose. By week 8, there was significant (p<0.05) recovery in GTP activity for all doses of lactic acid. A similar trend was observed for LDH activity.

Discussion

Both surgery and lactic acid treatments caused reduced appetite, abnormality in gait and dullness in some experimental goats. These responses are some of the behavioral indicators of a stressful or painful experience[15]. Behavioral indices of pain have also been reported in dogs castrated using zinc gluconate 7 days after treatment[16]. In

Table 6: Changes in seminal characteristics following intratesticular injections

Treatment	Volume (ml)		Colour			Motility			Concentration		
	Before	After	AQ	ML	CR	PR	GD	VG	NOR	OLG	AZO
Control	0.95	0.97	20	40	40	0.0	40	60	100	0.0	0.0
1ml/10kg	0.98	0.57*	93	7	0.0	11	0.0	0.0	0.0	11	89.0
2 ml/10kg	0.98	0.49*	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
3 ml/10kg	0.96	0.42*	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100

Except for volume, the figures represent percentages of goats in a category. For colour, AQ = aqueous ML = milky and CR= creamy; for motility, Pr = poor, GD = good and VG = very good, for concentration, NOR= normospermia (=2.21 x 109 cells/ml), OLG = oligospermia (= 0.02 x 109 cells/ml) and AZO = azoospermia (no cells). *Diminution in seminal volume after treatment; control = saline group
Before = semen volume 7 days before treatments; After = semen volume 28 days after treatments.

Table 7: Treatment-time effects on GTP /LDH activities

Treatment	Effect	GTP activity		LDH activity	
		Estimate	P-Value	Estimate	P-Value
Intercept		9.43	<0.0001	73.71	<0.0001
Lactic acid 1ml/10kg	Treatment	-4.85	<0.0001	-18.70	<0.000
Lactic acid 2ml/10kg	Treatment	-7.74	<0.0001	-38.80	<0.0001
Lactic acid 3ml/10kg	Treatment	-9.18	<0.0001	-61.55	<0.0001
Lactic acid 1ml/10kg	Time*Treatment	0.27	0.0297	2.26	<0.0001
Lactic acid 2ml/10kg	Time*Treatment	0.71	<0.0001	1.18	0.1530
Lactic acid 3ml/10kg	Time*Treatment	0.75	<0.0001	0.80	0.3294

All treatments significantly ($P < 0.05$) decreased GTP and LDH activities but activities increased with time after treatment. The estimates are REML

the present study, percentages of animals that showed behavioral stress responses were higher in the lactic acid treatment groups. A similar observation has also been made earlier in calves[11]. The pain-related behavioral responses in this study were positively correlated to doses of lactic acid. Such dose-dependent responses in goats were similarly reported following intratesticular injection of calcium chloride for castration[8]. The degree of tissue degeneration and systemic response correlates to chemical concentration[17].

The study revealed that weight gain and body condition scores of treated goats were not adversely affected except for very high doses of lactic acid. This is an indication that intratesticular injection of lactic acid at an appropriate dose could be a suitable alternative to surgery. Experiments with calcium chloride in black Bengal goats in India also lead to a similar observation[8].

Both surgery and lactic acid treatments caused elevation in leukocyte counts. Tissue destruction irrespective of cause will produce an elevation in number of circulating leukocytes[18]. The leukocyte response was more prolonged in the lactic acid treated animals than in surgery group. Cortisol responses also showed a similar trend. A similar trend was reported in calves castrated by intratesticular injection of lactic acid[11]. They reported similar pain intensity but longer pain duration in lactic acid treated

calves.

Surgery and lactic acid treatments caused elevations in plasma FSH levels, an indirect indication of reduced testicular function. Glycerol was observed to produce similar effects in rats intratesticularly injected[19]. Increased plasma FSH level is indicative of gonadal dysfunction due to reduced negative feedback mechanism of androgens on the gonadal-thalamic axis[20].

Ejaculate volumes dropped following intratesticular injection. This is in agreement with the general principle that reduced testicular activity affects mass and secreatory activities of accessory sex glands[16]. In dogs castrated using zinc gluconate, the prostate was observed to shrink by up to 52% in addition to reduced seminal volume[16]. Additionally, almost all treated goats in the current study had watery semen in comparison to the control group. Normal semen is typically cream or milky in appearence[21]. Thick, milky semen is good while amber or thin watery semen is evidence of low sperm concentration[22].

Mass motility was not detectable at higher doses of lactic acid. Earlier studies with other chemosterilizing agents showed that maximum responses in biochemical, histological and seminal parameters occur at higher doses[8,19]. Severe testicular degeneration at higher doses and volumes of lactic acid solutions apparently caused complete cessation of spermatogenesis and sterility in treated goats. *Azoospermia* and extreme oligospermia are measures of male infertility[16]. In an experiment with glycerol, an overall suppression of spermatogenesis, with about 90% fewer sperm in the epididymis was reported[19]. Like in this study, suppression in sperm production corresponded to dose.

Activities of GTP and LDH were suppressed suggesting loss of both Sertoli and germ cells, hence a non-specific necrotic effects of lactic acid. Unlike glycerol that causes selective suppression of the germinal epithelia[19], lactic acid appears to cause non-specific tissue necrosis. Calcium chloride, another sclerotising agent was observed to cause non-specific diminution in activities of both 3ß and 17ß hydroxysteroid dehydrogenase (HSD) in goats suggesting non-specific action on testicular tissue[8].

We conclude that, intratesticular injection of 88% lactic acid at appropriate doses ($\geq$ 2 ml/10kg body weight/testis) is effective for castration in adult goats. The method however, is responsible for a longer pain response than surgery hence may not be the best alternative in goats.

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EVALUATION OF RUMEN INGESTA BASED-DIET AS ALTERNATIVE FEED STUFF FOR GROWING RABBITS

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EVALUATION DE LA RATION A BASE D'INGESTA DU RUMEN COMME ALIMENT ALTERNATIF POUR L'ELEVAGE DES LAPINS

Résumé

Trente lapins croisés (lapin blanc de la Nouvelle Zélande x race locale) ont été utilisés dans une expérimentation pour évaluer l'effet des rations à base d'ingesta du rumen sur la performance des lapins et sur les propriétés sensorielles de leur viande. Trois rations alimentaires: ingesta du rumen (RI) séchés au soleil ; aliment pour volaille sous forme de pâtée (GM) et un mélange de RI et de GM (ratio 2:3 de RI /GM) ont été servies aux lapins. La consommation alimentaire, le gain de poids et la qualité de la carcasse ont fait l'objet d'évaluation. Il y avait une différence significative quant à la consommation alimentaire entre les lapins nourris de RI & GM et ceux nourris de GM ($p < 0,05$). Les lapins servis de RI & GM avaient un gain de poids nettement ($p<0,05$) inférieur (10,6g/jour) à ceux servis de GM (16,8g/jour). Il n'y avait pas de différence significative ($p>0,05$) entre les morceaux de viande de lapin et la ration alimentaire pour toutes les propriétés sensorielles évaluées par un groupe de huit membres utilisant une échelle Hédonique en dix points. On a isolé *Citrobacter diversus* des ingesta du rumen, des échantillons d'intestins et de bile de lapins dans tous les groupes. La viande de lapin du groupe nourri de RI & GM était moins chère et acceptable pour la consommation humaine. Dans le contexte de recyclage de nutriment surtout en milieu urbain, les déchets d'abattoir peuvent être réintégrés sans risque dans la chaine alimentaire sous forme de valeur ajoutée.

Mots-clés: Lapin, ingesta du rumen, gain de poids, qualité de la carcasse, propriétés sensorielles.

Summary

Thirty cross-bred rabbits (New Zealand white x local breed) were used in an experiment to evaluate the effect of rumen ingesta based diets on the performance of rabbits and on sensory properties of its meat. Three diets: sun-dried rumen ingesta (RI), poultry growers mash feed (GM) and mixture of RI and GM (RIGM ratio of 2:3) were fed to the animals. Feed intake, weight gain and carcass quality were assessed. There was significant difference in feed consumption between rabbits fed RIGM and those fed GM,

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($p < 0.05$). Rabbits fed RIGM showed significantly($p<0.05$) lower weight gain (10.6g/day) than those fed GM (16.8g/day). There was no significant($p>0.05$) difference between cuts of rabbit meat and feed regime for all sensory properties evaluated by an eight member trained panel using a ten-point Hedonic scale. *Citrobacter diversus* was isolated from rumen ingesta, intestinal and bile samples of Rabbits in all the groups. Rabbit meat from group fed RIGM was cheaper and equally acceptable for human consumption. In a nutrient recycling context especially in urban locations, abattoir waste can be safely re-integrated into the food chain in a value added form.

Keywords: Rabbit, Rumen ingesta, Weight gain, Carcass quality, Sensory Properties.

Introduction

Rabbit has emerged as one of the cheap sources of animal protein in the tropical zone[1]. This is mainly because of the cheap investment cost, easy adaptation to backyard management systems, high fecundity and resistance to many tropical diseases[2]. Rabbit production in the tropics remains relatively low due to scarcity of forages during dry season and high cost of conventional food stuffs like Maize and Soya beans[3]. The high cost of conventional feed-stuff is further complicated by the competition between man and livestock for these grains[4]. In developing countries rabbits are kept primarily for meat and it is being increasingly accepted nationwide as a ready cheaper alternative to poultry. Rabbit meat has high protein, low fat, low energy and cholesterol content[3].

FAO[5] has reported that there is an acute shortage of animal protein in the diet of most Nigerians. This is associated with low supply and high cost of the conventional meat and animal products. Current research efforts in most developing countries are therefore aimed at identifying potential feed sources that have little or no demand by humans. Such ingredient should be cheap and readily available for compounding livestock rations. Some of such potential feed materials that are being investigated include agro-industrial by-products, wastes from cattle ranches, abattoirs and poultry litter[6,7]. Abattoir waste (rumen ingesta) appears to be of great use in solving the problem of feed stuff for animals as its proper disposal is even a problem to the butcher and a serious threat to public health.

Therefore, in a nutrient recycling context, the abundance of rumen contents in the abattoir may provide needed alternative.

Sensory evaluation provides a measurable response to how much of an attribute or the intensity of a specific attribute, such as juiciness, tenderness or flavour is preferred. It also provides information on the acceptance or preference of the eating qualities of a product such as rabbit meat.

The aim of this study was to evaluate the effect of rumen ingesta based diets on the growth performance of rabbits and sensory properties of its meat.

Materials and Methods

Feed

Fourteen bags (560.0kg) of wet rumen ingesta were collected from fresh heaps of rumen contents within the premises of Zango abattoir Kaduna, spread on concrete floor

at the farm area of College of Agriculture and Animal Science, Ahmadu Bello University Kaduna, Nigeria and sun dried. The maximum temperature recorded throughout the eleven days' drying period was 40^{O}c and the lowest was 27.8^{O}c. The dry rumen ingesta was pulverized using mortar and pestle. The resultant material was bagged, labeled RI (Rumen ingesta) and stored for immediate feeding. Four bags (25.0kg each) of commercial poultry growers mash, (Guinea Feeds (R)) were emptied into a single bag, thoroughly mixed and labeled GM (Growers Mash). A 2:3 ratio was compounded from RI (19.6kg) and GM (58.8kg) by thoroughly mixing the two components on a disinfected concrete floor, bagged and labeled RIGM.

Experimental animals

Thirty cross-bred rabbits (New Zealand white x local breed) aged four weeks and weighing between 620g and 634g were randomly assigned to three groups (A, B and C). Each group of ten rabbits was housed in separate labeled hutches: The rabbits were conditioned for seven days by gradually introducing them to the experimental diets. At the end of the adaptation period, the rabbit were individually weighed and the weight recorded as the initial weight. Animals were offered daily 100.0g of each feed (RI, RIGM and GM) to groups A, B and C respectivey: Feed was offered every morning at 8.00am without any additional supplements. Water was available *ad libitum*.

Before each day's feeding, unconsumed feed was collected and weighed to determine the quantity consumed. The feeding period lasted 49 days. Daily mortality was recorded. The rabbits were weighed weekly.

Analysis of feed, faeces and organ samples

Simple floatation, sedimentation and modified Beerman's methods were separately used to examine for helminths and protozoa. Duplicate samples were collected in five animals of each experimental diet.

Samples (triplicate) of the experimental diets (RI, RIGM and GM), the small intestines, caecum, lungs, liver and bile of dead rabbits were separately inoculated in selenite and nutrient broth respectively at 37^{0}c overnight and sub-cultured onto blood agar and MacConkey agar. Isolates from each broth were subjected to following biochemical tests: triple sugar iron agar test, oxidation fermentation test, methyl red test, vogues-prousker and nitrate reduction test.

Proximate analysis was carried out to determine the dry matter (DM), crude protein (CP), ether extract (EE), Nitrogen free extract and Ash content of each, of the three feeds (RI, RIGM and GM) replicated three times. Three samples of carcass from each of the experimental groups were also analyzed for moisture (dry matter), fat and protein. Proximate analysis of the feed and meat samples from shoulder, back, loin and thigh muscles were analyzed using the methods described by AOAC[8].

Necropsy

Post mortem examination was carried out on all rabbits that died during the experiment. The organs were grossly examined while scrapings from the duodenum, jejunum, ileum, caecum and colon were examined microscopically for Coccidia oocysts.

Carcass Quality

At the end of the 7th week of test feeding, three rabbits from groups B and C were

slaughtered according to the method described by Cheeke[9] (all the rabbits in group A died after week 2 of test feeding). The carcass was cut into seven major points representing four standard cuts: shoulders, backs, loin and thighs as described by Aduku *et.al*[10]. Each of the standard cuts was weighed and little chunks were collected for proximate analysis. All the major cuts were washed as per the experimental group, split into two equal parts with a knife saw and cooked in an aluminum pot by boiling over gas flame from a double gas cooker. Cooking salt (2.5g/Kg of meat) was added to the content of each pot and as the water cooked out after twenty five minutes, 145.0ml of water was added to each pot and cooking continued for another twenty five minutes until the meat was well cooked. This procedure was repeated for the other half but no salt was added. From each pot, the meat was removed from the broth, allowed to cool and weighed to determine the cooking loss. Each cut was de-boned, sliced into 10.0g chunks for sensory evaluation and placed in plastic plates labeled (RIGMS and GMS for meat cooked with salt and RIGMS– and GMS– for meat cooked without salt. These were chilled in the freezing chamber of a refrigerator overnight.

Sensory evaluation

Eight slices weighing 10.0 g each from the major cuts (shoulders, backs, loin and thighs) from the two feeding regimes cooked with salt (RIGMS and GMS) and cooked without salt (RIGMS– and GMS–) were made out from the plastic plates after thawing and steam warming at 37^0c in an aluminum pot. A total number of 128 meat samples were thus derived and put in separate coded aluminum foils and wrapped using randomized 2-digit coded numbers. The sensory evaluation was for four days, evaluating one major cut each day. Before the evaluation, thirty two coded samples from a particular cut representing the two feeding systems cooked with and without salt were placed in correspondingly coded white serving plastic plates and served.

An eight member trained sensory panel selected randomly through a questionnaire evaluated the samples. Two members had previous taste panel experience. Two training sessions were used to familiarize panelists with the scoring system using a typical ten-point Hedonic Scale11 with 10 representing excellent, 5 representing indifferent and 1 representing very bad. Colour, taste, juiciness, flavour, tenderness, chewiness and overall acceptability were evaluated. Panelists were asked to integrate aroma and taste sensations to give flavour scores[12] and to determine juiciness during the finest chewing[13]. Tenderness was judged according to the ease with which the teeth sank into the meat when chewing began[13]. Chewiness was judged by the number of chews before the meat was swallowed. Tasting was carried out each day in the animal nutrition laboratory well lit with fluorescent tubes and devoid of distraction. The samples were served successively and all judges started evaluating at exactly 11.00 am. A maximum of 12 minutes and a minimum of 8 minutes were spent in evaluating each specimen. Each day's tasting lasted 48 minutes.

Data Analysis

Means and standard deviations were computed for the sensory scores. Analysis of variance (ANOVA) was used to determine significant difference between means among feeding regimes and their interactions for the sensory characteristics.

The analysis was done by using the General Linear Models (GLM) procedure of SPSS. Least significant difference (LSD) test was used in separation of means.

Results

Proximate analysis of feeds used in this study is presented in Table 1. There was no significant (P>0.05) difference in the chemical composition of the three rations (RI, RIGM and GM) used in this study except in their crude fibre content.

Table 2 showed the effects of the three feeding regimes (RI, RIGM and GM) on the growth performance of rabbits. All the rabbits fed RI died within 14 days of the experiment while no mortality was recorded in those fed RIGM and GM rations. The mean daily weight gain recorded in the rabbits fed RI diet within 7 days was 1.21g which decreased to 0.51g in the second week of the experiment before all the 10 rabbits gradually died. Also within the same period, the mean daily feed consumption of rabbits fed RI ration decreased from 29.4g to 24.1g before they completely went off feed and subsequently died. The daily weight gain for rabbits on RIGM was 7.9g within the first 7 days and 8.4g in the next 14 days. The daily feed consumption of rabbits fed RIGM increased from 51.6g in the first one week to 62.04g in the second week.

The daily weight gain for rabbits on GM was 12.0g in the first one week and 12.8g in the second week, whereas their daily feed consumption increased from 73.09g in the first week to 80.6g in the second week. The mean daily weight gain of rabbits fed RIGM was significantly (P<0.05) lower than 16.7g ± 3.4 recorded for rabbits fed GM. This was also the case with the mean daily feed consumption.

The proximate composition of meat from the two feeding(RIGM and GM) regimes is shown in Table 3. The water, protein and fat composition of meat from the two feeding regimes were not significantly different

Table 1: Means and Standard Deviations of Chemical Composition of Three Rations Used In Rabbit Feeding

	FEED		
Parameters	**RI***	**RIGM****	**GM*****
Nitrogen	2.8+0.0[a]	2.9±0.1[a]	3.1±0.1[a]
Ether extract	4.2±0.2[a]	3.0±0.0[a]	1.4±0.0[a]
Crude fibre	27.2±0.1[a]	22.4±0.1[a]	17.7±0.1[b]
Ash	10.7±0.1[a]	12.6± 0.1[a]	14.8±0.2[a]
Crude protein	17.5±0.1[a]	18.0±0.3[a]	19..1±0.3[a]
Dry matter	90.0±0.0[a]	89.4±0.3[a]	88.3 ±0.2[a]

* Rumen Ingesta

** 40% Rumen Ingesta + 60% grower mash

***100% Grower mash

a. Means with same superscript in row are not significantly different (P>0.05).

Table 2: Effect of 2 Feeding Regimes on the Growth Performance of Rabbits

Performance Parameters	Feeding Regime			Remarks
	RI	RIGM	GM	
Number of Rabbits	10	10	10	
Mean Initial Weight (g)	627.2 ± 3.3	630.7 ± 2.0	629 ±.1 3.6	
Mean Final Weight (g)	644.4 ± 3.4	1150.8 ± 18.7	1444.3 ± 3.7	
Mean weekly weight Gain (g)		74.2 ± 17.9	116.6 ± 23.5	P < 0.05
Mean Daily Weight Gain (g)	-	10.6 ± 2.6	16.7 ± 3.4	P < 0.05
Mean Weekly Feed Consumption (g)		458.9 ± 65.0	582.5 ± 43.5	P < 0.05
Mean Daily Feed Consumption (g)	-	65.6	81.2	P < 0.05
Mean Feed Conversion Ratio	-	6.2	4.9	
Feed Efficiency/Utilisation		0.16	0.2	
Growth Rate (%)		11.0	18.5	
Mortality		0	0	

Table 3: Means and Standard Deviations of the Chemical Composition of Rabbit Meat from Two Feeding Regimes

Factors (%)	Feeding Regimes	Whole Meat	Standard Cuts			
			Shoulder	Back	Loin	Thigh
Fat	RIGM*	7.7±0.1[a]	7.7±0.1	7.7±0.1	7.6±0.0	7.7±0.1
	GM**	8.0±0.0[a]	7.8±0.0	8.2±0.0	7.9±0.0	8.0±0.0
Protein	RIGM	19.8±0.0[a]	19.8±0.0	19.7±0.1	19.8±0.0	19.7±0.1
	GM	20.4±0.1[a]	20.7±0.1	21.0±0.0	20.0±0.1	20.0±0.1
Water	RIGM	72.1±0.8[a]	72.0±0.0	71.9±0.1	72.1±0.8	72.5±0.2
	GM	70.5±0.7[a]	70.5±0.7	69.8±0.4	71.8±0.0	70.0±0.0

* 40% Rumen Ingesta + 60% grower mash

** 100% Grower mash

a. Means with same superscript in row are not significantly different (P>0.05).

Note: The feed composition is on a dry matter basis.

(P>0.05). However, the meat from rabbits fed RIGM was lower in both protein (19.8%) and fat (7.7%) and higher in moisture content (72.1%) compared to meat from rabbits fed GM ration.

The mean values of the overall sensory properties evaluation for the whole meat and various cuts of rabbit meat from the two feeding regimes cooked with salt and without salt are presented in Table 4. The scores for the sensory properties showed that there was no significant difference (P>0.05) between the whole meat and the cuts among the two feeding regimes for both rabbit meat cooked with salt and without salt. However, the score values for cuts were

Table 4: Mean values of overall sensory scores for various cuts of rabbit meat from two feeding regimes cooked with salt and without salt

Cut	Type of Feeding			
	Cooked with salt		Cooked without salt	
	RIGM*	GM**	RIGM^{-}	GM^{-}
Shoulders	7.3±1.4	8.1±1.3	6.6±1.4	7.4±1.4
Back	6.9±1.4	7.7±1.5	6.4±1.4	6.6±1.4
Loin	6.2±1.4	7.0±1.9	6.0±1.4	6.5±1.6
Thighs	7.2±1.5	8.3±1.2	6.5±1.4	7. ±1.3

* 40% Rumen Ingesta + 60% Growers Mash
** 100% Growers mash

Table 5: Comparisons of meat from two feeding regimes with respect to sensory properties

Feeding Comparisons	Colour	Taste	Juiciness	Flavour	Tenderness	Chewiness	Overall Acceptability
Score mean	7.4	6.9	7.0	6.9	6.9	7.0	7.2
MSE*	1.7	2.0	2.1	2.1	1.9	2.0	2.0
LSD**	0.3	0.3	0.4	0.4	0.3	0.3	0.3
GMS Vs GMS^{-}	+	+	+	+	+	+	+
GMS Vs RIGMS	+	+	+	+	+	+	+
GMS Vs RIGMS^{-}	+	+	+	+	+	+	+
GMS^{-} Vs GMS	+	+	+	+	+	+	+
GMS^{-} Vs RIGMS	+	-	-	-	-	-	-
GMS^{-} Vs RIGMS^{-}	+	+	+	+	+	+	+
RIGMS Vs GMS	+	+	-	+	+	+	+
RIGMS Vs GMS^{-}	+	-	-	-	-	-	-
RIGMS^{-} Vs GMS	+	+	+	+	+	+	+
RIGMS Vs RIGMS^{-}	+	+	+	+	+	+	+
RIGMS^{-} Vs GMS	+	+	-	+	+	+	+
RIGMS^{-} Vs RIGMS	+	+	+	+	+	+	+

\+ = Higher Significant (p<o.05)
\- = Not significant (p>0.05)
Df Degree of freedom = 493
* Mean standard error
** Least significant difference
GMS Meat from rabbits fed Growers mash and cooked with salt
GMS^{-} Meat from rabbits fed Growers mash and cooked without salt.
RIGMS Meat from rabbits fed 40% Rumen Ingesta + 60% Growers mash cooked with salt
RIGMS^{-} Meat from rabbits fed 40% Rumen Ingesta + 60% Growers mash cooked without salt.

Table 6: Cost of Production of Rabbits from three rations in US Dollars ($)

ITEM	RIGM	GM
Feed cost	0.80	1.33
Labour cost	0.63	0.63
Transport cost	0.15	0.10
Total	1.58	2.06

comparatively higher for those cooked with salt for the two experimental groups and in favour of GM ration.

Comparisons of meat from two groups with respect to sensory properties are presented in Table 5. The mean scores for colour, taste, juiciness, flavour, tenderness, chewiness and overall acceptability of the rabbit meat from the two feeding regimes as evaluated by the panelists were 7.4, 6.9, 7.0, 6.9, 6.9, 7.0 and 7.2 respectively.

Table 6 showed the cost of production of rabbit from two rations (RIGM and GM) in US Dollars ($). The cost of production of rabbits on RIGM ration up to seven (7) weeks was 1.58 US Dollars while that of rabbit on GM ration was 2.06 US dollars.

Parasitological evaluation of feeds (RI, RIGM and GM), fecal and organ samples of both live and dead rabbits did not reveal any parasites. From microbiological evaluation, rough flat colonies of Gram negative bacteria were isolated from RI, RIGM and GM and organ samples such as the small intestine, caecum, lungs, liver and bile incubated in selenite broth and nutrient broth sub cultured on to MacConkey agar.

The enteric organisms identified were *Citrobacter diversus, Escherichia coli* and *Enterobacter aerogenes* all of which are common in the gastrointestinal flora. *Citrobacter diversus* was the main isolate from the intestines and bile.

Discussion

RIGM was higher in crude fibre (22.4%). The rabbits on this ration were expected to consume more of the RIGM to compensate for their energy requirements[14], but in this study, the GM group of rabbits (crude fibre 17.7%) consumed more feed; probably because GM was more palatable. The composition of the rumen ingesta used in this study differs from values reported in a previous study[15]. The higher values obtained in the present study could be due to the quality of feed consumed by the animals before slaughter. The crude fibre level recorded for RIGM was considered high since Crude fibre beyond 20% may cause caecal impaction[16]. Cases of caecal impaction or clinically ill animals were however not observed in this study.

The low fat level of 3.0% for RIGM may have decreased its palatability in accordance with the observation of German and Dillard[26]. The drop in both the mean daily feed intake, weight gain and mortality in RI ration could have been due to non-palatability and low efficiency of utilization of the RI feed. The seven days gradual introduction of a new

ration (100% RI) may not have been enough adaptation period for such early weaned rabbits (4 weeks of age) and could have contributed to the death recorded in the group. The isolation of enteric bacteria of normal gastro-intestinal flora in all the rations, feacal and organ samples of rabbits may have been enough reason to exclude infection as the cause of mortality recorded in the group fed RI diet.

The mean average feed intake of 65.6g/day for RIGM was close to 67.0g/day and 68.0g/day for rabbits fed cowpea haulms diet[10] and poultry waste[18] respectively. The average weight gain for GM (16.7g/day) was higher than the 12g/day previously reported by Ekpenyong[27] with weaning rabbits fed broiler starter mash and 9.5g/day for those fed grower mash. The weight gains obtained in this study could have been better if the feeds were pelleted because weight gain of rabbits on pelleted feed (44g/day) is significantly better than those on un-pelleted feed (29.3g/day)[28].

The fact that no mortality was associated with feeding of RIGM and also that the resultant weight gain compared favourably with that of GM showed that RI used at 40% level of inclusion met the growth requirements of the rabbits.

The values of water, protein and fat recorded in the rabbit in this study compared favourably with protein (18.6–19.4%), fat (1.9-10.9%) and water (68.5 –72%) obtained by Omojola, and Adesehinwa[29] and protein (22%), fat (5.4%) and water (71.5%) obtained by Polak *et al*[20]. Information on carcass quality and composition of rabbit meat is limited in contrast to that available for other types of meat.

Score values for cuts were comparatively higher in cuts cooked with salt for the two feeding regimes and in favour of GM. This can be explained by the taste of the meat cooked with salt as the taste receptors respond rapidly to a chemical stimulus. McCurdy[12] reported that sensory differences between feeding regimes were more pronounced than between breed types.

The mean score (7.4, moderately attractive) for colour is considered high since the human eye has a remarkably fine qualitative discrimination for colour though it is not a quantitative instrument. Colour and other aspects of appearance influence food appreciation and quality especially by the consumer. Flavour and juiciness may have been affected by cooking procedure as observed by McCurdy[12], that lower temperature reduces flavour and juiciness. The cost of production of rabbits fed grower mash is higher than the costs for RIGM. The variation in the costs of production for the two groups is mainly due to cost of feed as rumen ingesta is offered without cost in the abattoir.

According to Jawetz *et al.*[30], *C. diversus* is a member of the normal intestinal flora and is not strictly pathogenic. *C. diversus* could not have been strictly responsible for the mortality since it was also isolated from all the feed samples.

Conclusion

The low cost of production and close growth performance achieved through the use of RIGM as alternative feed for rabbit production is of economic advantage to the rabbit farmers. The use of RIGM as alternative feed in rabbit production will help rabbit farmers reduce the production costs and maximize profit especially during dry season period of feed scarcity. Rabbit meat from the group fed RIGM was, therefore, cheaper and equally acceptable for human

consumption.

Recommendation for the use of rumen ingesta as feed raw materials will also be of assistance in ridding the abattoir environment of heaps of rumen wastes thus minimizing environmental pollution and public health risks.

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SUPPLEMENTATION OF GRAZING CATTLE: THE USE OF A DIET CONTAINGING GRADED LEVELS OF DRIED CASHEW PULP

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COMPLEMENT ALIMENTAIRE POUR LES BOVINS AU PATURAGE: UTILISATION D'UN REGIME CONTENANT DES NIVEAUX CROISSANTS DE PULPE DE CAJOU SECHEE

Résumé

L'objet de la présente étude était de mettre au point des rations alimentaires pour le bétail pendant la saison sèche à la sous-station de Bole de l'Institut de Recherche sur le Cacao du Ghana (CRIG), en vue d'atténuer les pertes de poids durant la saison sèche, rations à base de gousse de cacao (CPH) et d'autres aliments pour bétail disponibles sur place tels que la pulpe de cajou séchée (DCP). On a utilisé vingt paires de vaches et de veaux selon un dispositif expérimental complètement randomisé. La pulpe de cajou séchée (DCP) a été servie à raison de 15% (traitement 2) ; 20% (traitement 3) et 25% (traitement 4). La ration-témoin (pas de complément alimentaire) a été servie comme traitement 1. L'essai a été mené pendant deux saisons sèches consécutives. Il n'y a pas eu de différences significatives entre les traitements en termes de taux de croissance des vaches et des veaux, et de consommation de matière sèche pendant la première année. Durant la deuxième année, toutefois, le traitement 1 était nettement différent (P <0,01) des traitements 2, 3 et 4 en termes de taux de croissance des vaches. Le taux de croissance des vaches était en moyenne de 0,050 ; 0,035 ; 0,018 et -0,129 kg jour^{-1} pour les traitements 1, 2, 3 et 4 respectivement. On en a conclu que 25% DCP pourraient être incorporés avec succès dans une ration complémentaire pour l'alimentation du bétail pendant la saison sèche.

Summary

The objective was to develop dry season feeding rations for cattle at the Cocoa Research Institute of Ghana (CRIG) Bole substation, to alleviate dry season weight loss, based on cocoa pod husk (CPH) and other, locally available feed ingredients such as dried cashew pulp (DCP). Twenty cow and calf pairs were used in a completely randomized design. Dried cashew pulp (DCP) was fed at 15% (treatment 2), 20% (treatment 3) and 25% (treatment 4). The control (no supplementation) served as treatment 1. The trial was run over two consecutive dry seasons. There were no significant differences among treatments (P > 0.05) in terms of cow and calf growth rate and dry matter feed intake in year 1. In year 2, however, treatment 1 was significantly different (P < 0.01) from treatments 2, 3 and 4 in terms of cow growth rate. Mean cow growth rate was 0.050, 0.035, 0.018 and -0.129 kg day^{-1} for treatments2, 3, 4 and 1, respectively. It was concluded that up to 25% DCP could be incorporated successfully in a dry season supplementary ration for cattle feeding.

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Introduction

The poor quality of forages in the dry season is generally well known[1,2,3,4,5,6]. During the dry season, therefore, cattle are unable to meet their requirements for maintenance and production. Animals are often in negative energy and nitrogen balance and as a result mobilize body reserves in a bid to survive. This is reflected in low growth rates/weight loss and reduced milk yield during the dry season, which ultimately affect the total output by the animals[3,4,5,6],. Attempts have been made to solve the dry season feed problem through the conservation of forage as hay[7,8] or silage[9,10,11]. Difficulties posed by uneven and unstumped grassland, lack of mowing implements, impossibility of cutting and transporting heavy bulky material by human labour alone combine to place these methods beyond the grasp of the village farmer at this present time.

Supplementation of grazing animals has been proven to be more efficient in improving animal productivity during the dry season. Various supplements (crop residues, browse plants, agro-industrial by-products,) have been tried with some success[12,13,14].

The Cocoa Research Institute of Ghana (CRIG) has an abundance of agro-industrial by-products such as cocoa pod husk and cashew pulp. Cashew pulp is the sun-dried residue after the juice has been extracted from the cashew apple and chemical analysis of cashew pulp (table 1) indicated that it is similar to cocoa pod husk, in terms of crude protein, and less fibrous and could be used for feeding animals. This paper reports on attempts being made to develop suitable dry season rations for cattle on the CRIG Substation at Bole in the Northern Region, based mainly on by-products generated internally by CRIG.

Materials and methods

Site

The study was carried out at the Bole Substation of the Cocoa Research Institute of Ghana (2^0 02' N; 2^0 29' W; 301). The substation experiences a dry period from November to April each year with high temperatures. Mean annual rainfall is 1087mm with a mean temperature of 26.1^{0}C.

The herd is basically managed under a pastoral system. The herd is predominantly West African Shorthorn, with a few Sanga

Table 1. Chemical composition of dried cashew pulp.

Dry matter (g Kg-1) g Kg^{-1}DM	866.2
Organic matter	951
NDF	260.8
ADF	49.6
ADL	34.2
Hemicellulose	211.2
Calcium	3.2
Phosphorous	0.5
N x 6.25	6.47

(cross between Zebu-type animal and West African Shorthorn). The herd leaves the station at about 9.00am in the morning and returns at about 4.00pm in the afternoon. These times are adhered to regardless of season. No milking is practiced and milk is left entirely to the calf. Animals are watered from a drinking trough, built for the purpose, on their way to and from grazing. Grass species in the grazing areas include *Andropogon gayanus*, *Hyparrhenia rufa*, *Imperata cylindrical*, *Brachiaria jubata* and *Panicum* species. Shrubs such as *Acacia nilotica* and *Cassia* species as well as *Centrocema* species (pasture legume) are also present. The shea tree (*Vitellaria paradoxa*) grows wild and is found scattered all over the grazing lands. Other management practices like endoparasite (Albendazole drench) and ectoparasite (spraying with acaricide) treatment were carried out for the whole herd. Routine vaccinations were given when due.

Experimental design and analysis

The trial was conducted as a completely randomised design with 4 treatments and 5 replicates (animals) per treatment making a total of 20 experimental units. Twenty cow and calf pairs (West African Shorthorn) were used for the experiment and were randomly allocated to the treatments. The cows (Year 1: 198.5 ± 32.6 kg; Year 2: 204.3 ± 29.7 kg) were in their second or third lactation and calves (Year 1: 25.1 ± 10.0 kg; Year 2: 28.3 ± 8.6 kg)were about three (3) months old. The experiment was run over two dry seasons, that is, from December 2004 to April 2005 (year 1) and repeated from December 2005 to April 2006 (year 2). December 2004 and December 2005 served as adjustment periods with data being collected from January to April in 2005 and 2006, respectively. Different animals were used in the two years during which the experiment was run. Analysis of variance was carried out, on each year's results, to

Table 2. Composition of experimental diets

	-----% of diet----		
INGREDIENT	**T2**	**T3**	**T4**
Cocoa pod husk	50	50	50
Cashew apple pulp	15	20	25
Fishmeal	5	5	5
Wheatbran	21.5	16.2	10.8
Urea (fertilizer grade)	2.5	2.8	3.2
Salt	5	5	5
Oyster shell	1	1	1

test the effects of the various treatments on cow and calf weight gain and also on dry matter intake of supplement by cows. The initial weight of cow and calf were used as covariates in the analysis of variance for cow and calf growth rate, respectively. Where there was a significant difference ($P < 0.05$), means were separated by the Least Significant Difference method (LSD)[15] The analysis was done using Genstat[16].

Supplements and their feeding

The fifteen cows receiving supplementation were individually fed in pens constructed for the purpose. They were fed their supplement in the morning, before grazing and in the evening after grazing. The supplements consisted of a mixture of cocoa pod husk and dried cashew pulp, with other ingredients being added to balance them (see table 2). After the extraction of juice from the cashew apple using a screw press, the residue or pulp is sun-dried on a concrete surface. Drying is completed in a day or two and the resulting material is termed dried cashew pulp. Dried cashew pulp was included at 15% (treatment 2), 20% (treatment 3) and 25% (treatment 4). Tuna meal was included in all diets (5%) to provide some rumen un-degradable protein. Treatments were made as iso-nitrogenous as possible. One kilogram per animal per day of the supplement was fed, half in the morning before grazing and the other half on the return of the animals from grazing. Refusals were collected before feeding, each morning, and weighed to determine supplement dry matter intake. The control (no supplementation) served as treatment 1.

Data collected were initial weight of cow and calf, monthly weight of cow and calf and dry matter intake of supplement per cow for the cows receiving supplements.

Laboratory analysis

All feeds were oven dried at 60^0C in a forced draught oven for determination of dry matter. Feeds were then milled through a

Table3. Chemical composition of experimental feeds

PARAMETER	**T2**	**T3**	**T4**
Level of dried cashew pulp -	15%-	-20%-	-25%-
Dry matter (g Kg^{-1})	905	825	885
g Kg^{-1}DM			
Organic matter	820	820	850
NDF	388.5	391.2	373.8
ADF	307.4	318.1	345.4
ADL	121.7	138.8	149.5
Hemicellulose	81.1	73.1	28.4
Nitrogen x 6.25	164.4	169.4	163.1

1.0 mm screen and analysed for Nitrogen[17] and Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL)[18]. Organic matter was determined as the weight loss after ignition in a furnace at 500^{0}C for 3 hours. Hemi-cellulose was calculated as the difference between NDF and ADF.

Results

The ingredient composition of the experimental feeds is shown in table 2. Chemical composition of the experimental feeds is shown in table 3 and means for cow and calf growth rate as well as supplement dry matter intake are shown in table 4 for both years.

The diets were fairly comparable with organic matter ranging from 820 g Kg^{-1} to 850 g Kg^{-1}. Neutral detergent fibre ranged from 373 g Kg^{-1} to 391.2 g Kg^{-1} and crude protein (N x 6.25) from 163.1 g Kg^{-1} to 169.4 g Kg^{-1}.

There was no significant difference (P > 0.05) between treatments in any of the parameters measured for year 1 (2005). Cows on the control (treatment 1), however, showed a negative growth rate as compared to cows on treatments 2, 3 and 4 which showed a positive growth rate. In year 2 (2006), however, treatments 2, 3 and 4 which received supplementation showed a low positive cow growth rate which was significantly different (P < 0.01) from treatment 1 (control) which received no supplementation and showed a negative growth rate. Calf growth rate and supplement dry matter intake were not significantly different (p > 0.05).

Table 4. Means for cow and calf growth rate and cow dry matter feed intake

Parameters (kg day^{-1})	Treatment 1 (control)	Treatment 2 (15% cashew pulp)	Treatment 3 (20% cashew pulp)	Treatment 4 (25% cashew pulp)	SED	Significance
YEAR 1						
Cow growth rate	-0.011	0.136	0.232	0.160	0.0851	NS
Calf growth rate	0.231	0.222	0.258	0.214	0.0506	NS
Supplement dry matter intake	-	0.561	0.514	0.652	0.1191	NS
YEAR 2						
Cow growth rate	-0.129	0.050	0.035	0.018	0.0352	* * *
Calf growth rate	0.211	0.229	0.302	0.281	0.0388	NS
Supplement dry matter intake	-	0.461	0.507	0.381	0.0715	NS

Discussion

The benefits of supplementation of grazing, especially during the dry season, cannot be over-emphasized, and have been elucidated by several authors[19, 20, 21]. Apart from providing valuable nutrients in themselves, supplements may also influence the functioning of the rumen by alleviating a deficiency in microbial fermentation, e.g. nitrogen or sulphur and by improving the rumen environment by increasing the number of cellulolytic organisms to increase invasion and adhesion rate of new substrate[22] Supplements may thus increase the supply of microbial protein. The supplements fed in this experiment were designed to influence microbial digestion and also supply rumen undegradable protein (addition of tuna meal).

There was a paucity of information on the feeding of dried cashew pulp to cattle or other ruminant animals. However, Sundram[23] reported the replacement of groundnut oil meal with cashew apple waste at 10% in a concentrate fed to Gir cows. After 90 days average daily dry matter intake with and without cashew apple waste was 8.92 and 8.95 kg, respectively. Milk yield was 5.17 and 5.19 kg with and without cashew apple waste, respectively. There was no significant difference (P>0.05) between treatments.

Cow growth rates and supplement dry matter intakes were generally higher in year 1 as compared to year 2. This could have been influenced by several factors such as temperature, pasture quality and animal variability which were not measured. Because different animals were used in year 1 and 2, an analysis for year effect could not be carried out. Variation between readings within a treatment for cow growth rate, in year 1, were quite large leading to a large coefficient of variation and standard errors and may have led to a lack of significance (P = 0.085) between treatments.

In conclusion one may say that the results over the two years indicate that it is beneficial to supplement cows during the dry season and the three feeds tested are equally good. Dried cashew pulp may therefore form up to 25% of the supplementary feed for cattle in the dry season.

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EXTERIORISATION DES CHALEURS CHEZ LA BREBIS DJALLONKE

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EXTERNAL SIGNS OF HEATS IN DJALLONKE EWES

Summary

A study on the external signs of heats was carried out, in 14 ewes in natural and induced heats. Ewes were divided into two groups that is a control in natural pasture only and a supplemented group, which received a multi-nutrient supplement in addition to pasture feeding. Heats induction was done using Medroxy-progesterone Acetate and PMSG. The frequency of bellowing (6 / hour), and urination (7.50±0.50 /hour) was significantly influenced by the presence of the ram ($p<0.05$). Lifting of the tail was 34 % more elevated in supplemented ewes although not significant. Mounting of ewes by the ram was more frequent in the non supplemented ewes. Whatever the type of heats, the cervix was only open during oestrus and during which vagina was reddish, the vulva humid and turbid. Whatever the type of heats, the vaginal pH was more acid during estrus (6.48±0.30 on natural heats vs 6.83±0.41 on induced heats) and the elasticity was higher; the mucus was also abundant and transparent. In conclu-sion, parameters that can be considered as criteria for determining estrus are behavioral signs, the aspect of vulva, vaginal membrane and mucus, pH and elasticity of mucus, opening of the cervix.

Key words: Djallonke ewe, heats, oestrus, behaviour, temperature, pH, vaginal mucus

Résume

Un essai sur l'extériorisation des chaleurs a été réalisé chez 14 brebis, sur chaleurs naturelles et induites. Les brebis étaient réparties en deux lots dont un témoin et l'autre recevant un supplément multi-nutritionnel. L'alimentation de base était constituée de fourrages prélevés sur le parcours. L'induction a été faite à l'aide du médroxy-progestérone et de la PMSG. Les fréquences de bêlements (6/heure) et d'urination (7,50 ± 0,50 /heure) ont été significativement influencées par la présence du bélier ($P<0,05$). Le port de queue relevée a été de 34% plus élevé chez les brebis supplémentées. Quel que soit le type de chaleurs, le col de l'utérus n'est resté ouvert que pendant l'œstrus et au cours duquel les muqueuses vaginales étaient rougeâtres, la vulve humide et tuméfiée.

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Que ce soit sur chaleurs naturelles ou induites, le pH vaginal a été plus acide pendant l'œstrus (6,48 ± 0,30 sur chaleurs naturelles vs 6,83 ± 0,41 sur chaleurs induites); le mucus cervico-vaginal a été plus abondant et transparent, avec une élasticité plus élevée pendant l'œstrus. Les paramètres pouvant être retenus comme critères de détection de l'œstrus sont: les modifications comportementales, l'aspect de la vulve, des muqueuses et du mucus, le pH et l'élasticité du mucus, l'aspect du col.

Mots clés : brebis Djallonké, chaleurs, œstrus, comportement, température, PH, mucus vaginal.

Introduction

Dans les pays en développement, la consommation de viande est de 20g en moyenne par personne et par jour, soit 2 à 3 fois moins que les normes recommandées par la FAO. Cette faible consommation est due entre autres à une faible productivité du cheptel associée à une forte croissance démographique, d'où la nécessité de développer et d'améliorer la productivité de l'élevage.

Parmi les espèces susceptibles de contribuer à l'accroissement de la production et de la productivité de l'élevage figurent les petits ruminants. Ces derniers, notamment les moutons Djallonké jouent un rôle socio-économique très important. Ils servent dans les rites funéraires, les cérémonies culturelles et religieuses[1]. De plus, comparés à l'élevage du gros bétail (bovin), ils constituent un placement facilement mobilisable et une source de protéines appréciables. Un de leurs principaux intérêts réside dans la rusticité et surtout la trypanotolérance qui permettent leur élevage dans les zones forestières équato-guinéennes.

Dans l'optique de l'amélioration de la productivité, la maîtrise de la reproduction constitue un des objectifs majeurs, dans laquelle la détection des chaleurs joue un rôle capital. Mais il se pose généralement des problèmes d'anoestrus et de chaleurs silencieuses, particulièrement chez les races tropicales, qui ont été décrits chez la brebis Djallonké[2, 3]. En Afrique tropicale où les systèmes d'élevage sont majoritairement extensifs, les animaux sont très peu suivis et leurs performances dépendent de la capacité à s'adapter à leur environnement. De même, l'alimentation de ces animaux est essentiellement végétarienne, donc très souvent carencée en nutriments essentiels. Pourtant les conséquences d'un faible apport nutritionnel vont, selon l'intensité de la perturbation, d'une diminution du taux d'ovulation chez les espèces polyovulantes à une irrégularité des cycles voire un arrêt total de cyclicité. De même, la nature des aliments ingérés a des conséquences sur l'interface métabolisme/reproduction, une alimentation végétarienne affectant plus rapidement les cycles sexuels qu'une alimentation diversifiée [4].

Dans les années à venir, l'élevage en général et celui des petits ruminants en particulier est appelé à être intensifié avec une plus grande maîtrise des paramètres de reproduction. L'objectif du présent travail est de mieux connaître les manifestations d'œstrus chez la brebis Djallonké, ce qui permettrait de réduire le temps d'infertilité et le cycle de la reproduction chez les ovins.

Matériel et méthode

Présentation du milieu d'étude

Dschang est une localité des hauts plateaux de l'ouest Cameroun (L.N 5 - 7^0, et L.E 9 -12^0), où l'altitude oscille entre 1400 et 2100 m avec une moyenne de 1800 m, le climat est de type tropical soudano-guinéen et tempéré par l'altitude avec une saisons des pluies (de mars à octobre) et une saison sèche (de novembre à février). La pluviométrie varie de 1500 à 2000 mm, avec une humidité relative moyenne de 76% et une température moyenne annuelle comprise entre 18 et 21^0C. L'insolation moyenne annuelle est de 1848 heures. La végétation est caractérisée par une savane arbustive avec des galeries forestières dans les bas-fonds.

Matériel animal et conduite du troupeau

16 moutons (14 brebis et 2 béliers) de race Djallonké, âgés de 2 à 3 ans et achetés dans un marché local, ont été utilisés. Leurs poids variaient entre 14,78 et 25 kg. Pour chaque animal, l'âge a été estimé par l'examen de la dentition, suivant la méthode proposée par Gatenby[5].

Les animaux ont été au préalable étiquetés, vaccinés contre la peste des petits ruminants, soumis à un déparasitage interne et externe, et à une antibiothérapie de couverture à l'oxytétracycline. De plus, ces animaux on été tout au long de l'essai suivis et soignés chaque fois que nécessité s'imposait.

A la ferme, deux box de 9,30 m² chacun ont servi de logement aux brebis, les béliers étant logés dans un autre compartiment.

Pendant toute la durée de l'expérimentation, les brebis et les béliers étaient conduits séparément au parcours le matin entre 8 et 9 heures et ramenés à la bergerie entre 16 et 17 heures.

L'essentiel de l'alimentation était constitué de fourrages poussant sur le parcours et distribués le soir à l'auge, dominés par *Bracharia ruziziensis, Pennisetum purpureum, Trypsacum laxum, Bidens pilosa, Sporobolus sp, Hyparrhenia Cymboria*, *Setaria sp., Vernonia sp., Floribredul sp., Aspilia africana.* Le soir, 7 brebis de l'essai recevaient en plus, un supplément de bloc à nutriments multiples composé de Mélasse (30%), du son de blé (25%), de tourteau de palmiste (12%), d'urée (10%), de ciment (8%), de sel de cuisine (7%), de farine d'os (5%), de farine de coquillage (1%) et de chaux vive (2%). Le tableau 1 donne la composition chimique de ce bloc à nutriments multiples.

Tableau 1 : composition chimique du bloc à nutriments multiples

Bloc	Composition chimique (% Matière sèche)								
	MO	PB	Lipides	Cendres	NDF	ADF	ADL	Cell.	Hémicell.
BMMU	70,97	37, 43	1,00	29,03	25,03	19,68	14,92	4,76	5,35

BMMU: Block Multi Minéral à Urée, MO: Matière Organique, PB: Protéines Brutes, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, Cell: Cellulose, Hémicell.: Hémicellulose

Protocole expérimental

L'essai s'est déroulé en deux phases. Une première phase d'une durée de 24 jours (durée d'un cycle complet chez la brebis) a consisté en la détection des chaleurs naturelles, chez les brebis supplémentées et non supplémentées au bloc mulinutritionnel, à travers les modifications comportementales et les paramètres physicochimiques tels que la température vaginale, l'acidité et l'élasticité du mucus. L'aspect de la vulve (état de tuméfaction, couleur), l'aspect du col (ouvert ou fermé), l'abondance ou l'absence du mucus ainsi que son aspect (couleur, viscosité) étaient également enregistrés. La collecte du mucus a été rendue possible grâce à un spéculum introduit aussi profondément que possible dans le vagin.

L'observation des modifications comportementales était basée essentiellement sur le port de la queue, la fréquence d'urination et des bêlements, la posture en présence du mâle.

La température vaginale a été déterminée à l'aide d'un thermomètre à mercure. Le pH vaginal était mesuré à l'aide d'un pH- mètre électronique portatif en introduisant l'électrode aussi profondément que possible dans le vagin de la brebis. L'élasticité du mucus a été mesurée à l'aide d'un pied à coulisse précis au dixième de millimètre. Le mucus était récolté avec une spatule et déposé sur la mâchoire supérieure du pied à coulisse. En faisant coulisser les deux mâchoires l'une contre l'autre, on obtenait la valeur de l'élasticité au point de rupture du filament de mucus. Les prélèvements étaient effectués tous les deux jours.

La deuxième phase d'une durée de 14 jours a consisté en la détection des chaleurs induites chez les brebis supplémentées et non-supplémentées au bloc mulinutritionnel, et a porté sur le même échantillon de brebis utilisé à la première phase. L'induction s'est faite à l'aide d'éponges intra-vaginales à usage vétérinaire (Veramix®), contenant 60 mg d'acétate de médroxy progestérone. L'éponge a été introduite dans le vagin à l'aide d'un spéculum spécial recouvert de vaseline comme lubrifiant. Au retrait des éponges, 13 jours après, les brebis recevaient chacune 500 UI de gonadotropine sérique (Folligon®). Par ailleurs, les aspects du mucus et du col étaient relevés. Les fréquences de bêlement et d'urination ainsi que la posture des femelles étaient notées en présence et en l'absence du mâle. Par ailleurs, le port de queue et les chevauchements étaient notés.

Analyses statistiques

Les paramètres physico-chimiques et techniques ont été soumis à l'analyse de la variance. Lorsque les différences entre les paramètres étaient significatives, les moyennes ont été séparées par le test t de Student au seuil de 5%.

Résultats

Influence du type d'alimentation et de la présence du mâle sur la durée de l'œstrus et les modifications comportementales chez la brebis Djallonké en œstrus

Le tableau 2 présente la durée de l'œstrus ainsi que les modifications comportementales chez la brebis seule, en présence du mâle et en fonction de l'alimentation.

Modifications anatomo-fonctionelles

L'évolution des paramètres anatomo-fonctionnels avant, pendant et après les chaleurs naturelles et induites, est résumée au tableau 3.

Evolution de la température deux jours avant, pendant et 14 jours après les chaleurs naturelles chez la brebis Djallonké

Sur chaleurs naturelles, au moment de l'œstrus, les températures vaginales des brebis ont, indépendamment de l'alimentation, accusé une légère baisse de l'ordre de 0,67% par rapport à celles relevées deux jours avant, suivie au quatrième jour après l'œstrus, d'une baisse de 1,45% puis d'une augmentation de 3,32% entre le quatrième et le quatorzième jour après l'œstrus. Lorsque l'alimentation est considérée, les températures vaginales enregistrées chez les brebis supplémentées ont été en général supérieures à celles des brebis non supplémentées (Tableau 3).

Evolution de la température 48 heures avant, pendant et 24 heures après les chaleurs induites chez la brebis Djallonké

Sur chaleurs induites, Indépendamment de l'alimentation, les températures vaginales ont évolué en dents de scie. Les maxima ont été observés au moment de l'œstrus, 48 et 24 heures avant, et 24 heures après l'œstrus, tandis que les minima ont été enregistrés 36 et 12 heures avant, puis 12 heures après l'œstrus (Tableau 3).

Les températures observées chez les brebis supplémentées jusqu'au moment de l'œstrus ont eu une évolution opposée, comparée à celle des brebis non supplémentées. Les températures vagina-

Tableau 3 : Evolution des paramètres anatomo-fonctionnels avant, pendant et aprè l'œstrus.

	Paramètres	Temps (jours)								
	Température (°C)	**-2**	**0 (œstrus)**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Chaleurs naturelles	Tns	38,55*± 0,64	38,22* ±0,11	38,30*± 0,14	37,70*± 0,00	38,10*± 0,00	39,10*± 0,00	38,30*± 0,00	38,10*± 0,00	/
	Ts	38,81*± 0,81	38,57* ±0,25	38,21*± 0,13	38,00*± 0,71	38,73*± 0,45	38,92*± 0,30	39,05*± 0,87	39,19*± 0,86	39,2 ±1,13
	Moyenne	38,76^{ab} ±0,72	38,5^{ab} ±0,28	38,23^{ab} ±0,12	37,94^{a}± 0,63	38,57^{ab} ±0,49	38,96^{ab} ±0,27	38,9^{ab}± 0,83	38,97^{b}± 0,89b	39,2^{ab} ±1,13
	Aspect du col	F	O	F	F	F	F	F	F	F
		Temps (heures)								
Chaleurs induites	**Température (°C)**	-48	-36	-24	-12	0	12	24		
	Tns	38,60* ±0,57	39,30* ±0,28	38,20* ±0,28	39,05* ±0,21	38,82* ±0,04	38,30* ±0,57	39,20* ±0,00		
	Ts	39,30* ±0,00	38,30* ±0,44	39,53* ±0,23	38,67* ±0,21	39,06* ±0,23	38,97* ±0,23	39,00* ±0,00		
	Moyenne	38,95^{a} ±0,52	38,7^{a} ±0,64	39,00^{a} ±0,76	38,82^{a} ±0,28	38,97^{a} ±0,21	38,7^{a} ±0,49	39,1^{a} ±0,14		
	Aspect du col	F	F	F	F	O	F	F		

F = fermé ; O = ouvert, Tns = Température des brebis non supplémentées ; Ts = Température des brebis supplémentées

a,b : les chiffres sur les lignes portant les mêmes lettres ne sont pas significativement différents au seuil de 5%

* : les chiffres dans les colonnes portant le même nombre d'astérisque ne sont pas significativement différents au seuil de 5%.

les obtenues chez les brebis non supplémentées 36 heures avant l'œstrus, ont été significativement plus élevées (P<0,05) que celles relevées chez les brebis supplémentées.

Etat du col de l'utérus au cours du cycle

Quels que soient les traitements considérés, le col de l'utérus n'est resté ouvert qu'au moment de l'œstrus (Tableau 3).

Tuméfaction de la vulve et couleur des muqueuses vaginales

Quels que soit le type de chaleur, La tuméfaction de la vulve au moment de l'œstrus a été observée chez moins de deux tiers des brebis parmi lesquelles près de 70% des brebis supplémentées (Tableau 3). Par ailleurs, chez toutes les brebis, quel que soit le paramètre considéré, les muqueuses vaginales rougeâtres ont été observées pendant l'œstrus et rose-pâles en dehors de celuici. Ces modifications ont été mieux observées pendant les chaleurs induites.

Evolution des caractéristiques physico-chimiques du mucus cervico-vaginal au cours du Cycle chez la brebis Djallonké

L'évolution des caractéristiques du mucus en fonction du type de chaleurs et de l'alimentation est présentée au Tableau 4.

Quel que soit le type de chaleur (naturelles ou induites), l'alimentation et la période de la journée, le mucus a été transparent et abondant au moment de l'œstrus. Pour ce qui est de la couleur, le mucus a été blanchâtre deux jours avant et après les chaleurs naturelles ; au quatrième jour après l'œstrus, il est devenu caséeux, compact, puis a disparu totalement à partir du 6è jour après l'œstrus. Sur chaleurs induites, le mucus blanchâtre a été enregistré 24 heures avant et après l'œstrus. Lorsque l'on tient compte de l'abondance, ce paramètre n'a été observé que pendant, et 2 jours après les chaleurs naturelles et, lors des chaleurs induites, pendant et 12 heures après. Elle a été plus intense et plus évidente lors des

Tableau 4: Evolution des caractéristiques du mucus cervico-vaginal avant, pendant et après les chaleurs naturelles ou induites

Paramètres	*Temps (jours)*								
	-2	**0 (œstrus)**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Mucus CN									
- présence	+	+	+	+	-	-	-	-	-
- aspect	Blanchâtre	Transparent	Blanchâtre	Caséeux	/	/	/	/	/
- abondance	Peu	Abondant	Abondant	Peu	/	/	/	/	/
Elasticité CN	4,00^{a} ±0,12	9,59^{abc} ±6,97	3,20^{b} ±0,30	2,00^{c} ±0,00	/	/	/	/	/
	Temps (heures)								
	-48	**-36**	**-24**	**-12**	**0 (œstrus)**	**12**	**24**		
Mucus CI									
- présence	-	-	-	+	+	+	+		
- aspect	/	/	Blanchâtre	Blanchâtre	Transparent	Trouble	Blanchâtre		
- abondance	/	/	Peu	Abondant	Abondant	Abondant	Peu		
Elasticité CI	/	/	/	5,00^{a} ±0,02	10,6^{b} ±2,04	16,00^{b} ±7,07	2,00^{c} ±0,00		

- =absent ;+ = présent.
a,b : les valeurs sur les lignes portant les mêles lettres ne sont pas significativement différentes au seuil de 5 %
CN=Chaleurs Naturelles, CI= Chaleurs Induites

chaleurs induites (Tableau 4).

Evolution du pH 2 jours avant, pendant et 14 jours après les chaleurs naturelles chez la brebis Djallonké

L'évolution du pH avant, pendant et après les chaleurs naturelles est résumée au tableau 4, aussi bien chez les brebis supplémentées que non supplémentées. Dans le lot des brebis non supplémentées, le pH a subi une légère baisse de 2,58 % suivie d'un accroissement de 14,87% entre le quatrième et douzième jour. A l'inverse, chez les brebis supplémentées, une baisse brutale de 13,30% a été observée entre le 6è et le 8è jour après l'œstrus. L'analyse de la variance a montré une différence significative (P<0,05) entre le pH au moment de l'œstrus et sa valeur dix jours plus tard.

Evolution du pH 48 heures avant, pendant et 24 heures après les chaleurs induites chez les brebis Djallonké

L'évolution du pH avant, pendant et après les chaleurs naturelles est présentée au tableau 4. Indépendamment de l'alimentation, le pH a augmenté de 3,6% entre 48 et 24 heures avant l'œstrus, puis a amorcé une baisse de 3,94% et 6,05% respectivement au moment de l'œstrus et 12 heures après, comparée à sa valeur 24 heures avant l'œstrus. Lorsqu'on tient compte de l'alimentation, c'est dans le lot non supplémenté que l'on a observé les variations les plus grandes. De plus, le pH a évolué de manière opposée au moment de l'œstrus en fonction de l'alimentation. C'est ainsi que dans le lot non supplémenté, le pH a été le plus acide alors que chez les brebis supplémentées, il tendait plus vers la neutralité. Aucune différence significative (P<0,05) n'a toutefois été enregistrée.

Evolution de l'élasticité du mucus avant, pendant et après l'œstrus en fonction du type de chaleur

L'évolution de l'élasticité du mucus avant, pendant et après l'œstrus en fonction du type de chaleur et de l'alimentation est résumée au tableau 4. Indépendamment de l'alimentation et de la période de la journée, l'élasticité du mucus sur chaleurs naturelles a doublé pendant l'œstrus comparé à celle observée deux jours avant. Après l'œstrus, elle a diminué d'environ 66,63 % et 79,14 % respectivement deux et quatre jours après.

Pour ce qui est des chaleurs induites, l'élasticité du mucus est passée du double au triple respectivement pendant l'œstrus et 12 heures après, comparée à sa valeur 12 heures avant l'œstrus. La valeur la plus faible a été observée 24 heures après l'œstrus. Lorsqu'on considère le type de chaleur, l'élasticité sur chaleurs induites a été la plus élevée.

Discussion

Dans notre essai, nous avons enregistré chez les brebis Djallonké une durée du cycle de l'ordre de 17 ± 1,15 jours. Cette valeur se situe dans les limites mentionnées par Lhoste *et al.*[6] pour d'autres races ovines.

Pour ce qui est de la durée de l'œstrus, si les valeurs trouvées sont proches de celles relevées par Hounzangbé-Adoté[7], elles sont par contre inférieures de 31,25 à 54,17 % à celles obtenues par Boly *et al.*[8] chez les brebis Djallonké variété «Mossi». Les modifications comportementales et anatomo-fonctionnelles observées sont conformes à celles décrites chez d'autres races ovines[2,9]. Par ailleurs, nous avons observé une expression plus intense et plus évidente des manifestations de l'œstrus en

présence du mâle. Ceci serait dû aux phéromones mâles qui ont une influence sur le comportement sexuel des femelles[3]. Comme attendu, le col de l'utérus n'a été ouvert qu'au moment des chaleurs.

L'évolution de la température vaginale avant, pendant et après l'œstrus ne semble pas corrélée avec celui-ci, et ne pourrait par conséquent être un critère de détection de l'œstrus. Ce résultat a également été obtenu par Obounou[10] sur les brebis Texel. Les variations de la température observées entre le matin et le soir seraient liées à l'activité métabolique des animaux. En effet, les températures basses obtenues le matin ont été enregistrées sur des brebis à jeun et par conséquent à activité métabolique ralentie. Par contre, les observations du soir ont été faites sur des animaux à activité digestive intense, étant donné que la digestion s'accompagne d'une libération d'énergie sous forme de chaleur. Les différences observées entre brebis supplémentées et non supplémentées s'inscrivent également dans ce contexte.

Dans notre essai, il est apparu que le pH du mucus cervico-vaginal paraissait plus acide au moment de l'œstrus. Ce résultat est conforme à celui obtenu par Obounou[9] sur les brebis de race Texel. Cette tendance à l'acidification du mucus au moment des chaleurs pourrait être liée aux variations de sa composition chimique influencée par les œstrogènes. Ainsi le pH du mucus évoluerait sous le contrôle hormonal au cours du cycle sexuel. La chute brutale du pH enregistrée au 8è jour après l'œstrus chez les brebis supplémentées pourrait être associée à des facteurs environnementaux non encore identifiés. Par ailleurs, il semblerait que l'alimentation ainsi que le type de chaleur ait une influence sur ce paramètre. En effet, le pH le plus acide a été observé au moment des chaleurs naturelles et chez les brebis non supplémentées. Le pH plus acide observé 12 heures après les chaleurs induites pourrait s'expliquer par le fait que chez ces animaux, l'ovulation intervient à la fin de l'œstrus. Or c'est à ce moment précis que le taux sanguin d'œstrogènes atteint son niveau le plus élevé. Ainsi comme évoqué par Driancourt et Levasseur[11], les œstrogènes acidifieraient le mucus cervico-vaginal. La présence d'un mucus transparent, plus abondant et plus élastique pendant l'œstrus, a également été mentionnée par Vaissaire[2] et Obounou[10] chez d'autres races ovines (Texel). De plus, à des valeurs d'élasticité élevées correspondent des valeurs de pH plus acide.

Conclusion

Au terme de cette étude, les indicateurs des manifestations de l'œstrus chez la brebis Djallonké peuvent être regroupés en deux catégories : celles qui sont d'observation facile en conditions pratiques d'élevage (fréquences des bêlements et d'urination, port de la queue, chevauchement des brebis par le bélier, aspects de la vulve et celui du mucus) et celles qui nécessitent une certaine instrumentation et qui ne peuvent être utilisées qu'en conditions expérimentales (pH vaginal, ouverture ou fermeture du col, élasticité du mucus cervico- vaginal). Par ailleurs, la supplémentation des brebis au bloc multi-nutritionnel n'a pas eu d'effets perceptibles sur les paramètres étudiés. La réponse à l'induction semble indiquer que cette technique peut être valablement utilisée comme méthode de maîtrise du cycle sexuel chez la brebis Djallonké, même s'il faut encore attendre les résultats de leurs effets sur la fertilité.

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SHORT COMMUNICATION

RECTAL TEMPERATURE AND RESPIRATORY RATES IN TWO DIFFERENT NIGERIAN ZEBU BREEDS OF CATTLE

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Homeotherms can carry on their usual activities under a wide range of external temperature, by ensuring that the deep body temperature is maintained within a narrow limit[1]. This is done by either adjusting the rate of heat-gain or by heat–loss from the body, or adjusting the rate of generation of heat through various biochemical processes. Many conditions are capable of causing normal variations in the body temperature of cattle. These include age, sex, season, time of day, environmental temperature, exercise, eating, digestion, and drinking of water[2, 3]. It was also been reported that diurnal rhythm and œstrous cycle cause variations in the body temperature of domestic cattle[4].

Respiratory rate or frequency refers to the number of cycles or number of breaths taken each minute. It is an excellent indicator of health status but is influenced by many factors such as species, body size, age, exercise, excitement, environmental temperature, pregnancy, degree of filling of the digestive tract, and state of health. All domestic animals will increase frequency of breathing continuously as environmental temperature rises, thus aiding in thermoregulation. This often does not reach the stage of overt panting but can significantly increase alveolar ventilation and dead–space ventilation [2]. Although there are few reports on the rectal temperature (RT) and respiratory rates (RR) in the Zebu breed of cattle, there has not been any report on the effect of diurnal variation on these parameters. The aim of this study was therefore to determine their diurnal variation in the White Fulani cattle and Sokoto Gudali breeds of cattle.

Twenty-eight adult White Fulani and nine adult Sokoto Gudali cattle were used for the present study. They were treated against nematode parasites at 3 months intervals with Levamisole HCL (Levadex ®. Pantex, Holland B. V.) at 5mg/kg body weight, intramuscularly. They were grazed during the day on natural pasture for approximately 7 to 8 hours, which was supplemented by feeding a concentrate ration which consisted mainly of Brewers' Dry Grain., given after grazing. Clean water was supplied *ad libitum*. Measurement of RT and RR were taken at 08:00h, 15:00h, and 18:00h during the period of the study. The RT was recorded with the aid of a clinical thermometer (DT203 Digital Thermometer, OST Inc. China), which was inclined to make contact with the rectal mucous membrane, and was allowed to stay in the rectum of each animal for 2 to 3 minutes, when a beeping sound was heard, before the reading was taken. The RR in each animal was determined by counting the number of respiratory flank movements per minute. All data obtained were subjected to Student's

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Table 1: Effect of diurnal variation on the rectal temperature and respiratory rate of the White Fulani cattle (n = 28)

Time (hour)	Rectal temperature. (^{0}C)	Respiratory. Rate (breaths/minute)
0800	37.90 ± 1.59^a	20.04 ± 6.75^c
1500	39.06 ± 2.38^b	24.21 ± 7.89^d
1800	39.02 ± 2.31^b	22.02 ± 1.91$^{c\,d}$

Data with different superscripts in the same column are statistically different (P< 0.05).

Table 2: Effect of diurnal variation on the rectal temperature and respiratory rate of the Sokoto Gudali cattle (n = 9)

Time (hour)	Rectal temperature. (^{0}C)	Respiratory. rate (breaths/minute)
0800	37.93 ± 1.30^a	19.18 ± 2.95^c
1500	39.86 ± 1.32^b	21.57 ± 1.78^d
1800	39.06 ± 0.12^b	22.78 ± 4.32$^{c\,d}$

Data with different superscripts in the same column are statistically different (P< 0.05).

t–test. Values of P<0.05 were considered significant.

Meteorological data during the experimental period indicated that the period had a minimum temperature of 22.38± 0.59^{0}C and a maximum temperature of 31.53±4.87^{0}C. The Relative Humidity was very high (83.75± 2.88) throughout the period.

Table 1 shows the diurnal variation in the RT and RR of the White Fulani cattle. The rectal temperature of 37.90±1.59^{0}C at 08:00hr rose to 39.06±2.38^{0}C and 39.02±2.31^{0}C at 15:00h, and 18:00h, respectively. The RT at 08:00h was significantly lower than its values at 15:00h (P<0.01) and 18:00h (P<0.05), while the RT at 15:00h was similar to its value at 18:00h. The RR rose from 20.04± 6.75 breaths/minute to 24.21±7.89 breaths/minute, and decreased to 22.02± 1.91 breaths/minute at 08:00h, 15:00h, and 18:00h respectively. The RR at 08:00h was significantly lower (P<0.05) than its values at 15:00h, but similar to the value at 18:00h.The RR values at 15:00h and 18:00h were similar.

The RT of Sokoto Gudali cattle(Table 2) rose from its minimum value, 37.93±1.30^{0}C, to 39.86± 1.32^{0}C and 39.06± 0.12^{0}C at 08:00h, 15:00h, and 18:00h, respectively. The value at 08:00h was significantly lower than those at 15:00h (P<0.01) and 18:00h (P<0.05), while the values at 15:00h and 18:00h were similar. The RR rose from its lowest value, 19.18+2.95 breaths/minute at 8:00h to 21.57± 1.78 breaths/minute at 15:00h, and 22.78± 4.32 breaths/minute at 18:00h. The value at 08:00h was significantly lower

(P<0.05) than its value at 15:00h, but similar to that of 18:00h. The values at 15:00h and 18:00h were similar.

Pearson's correlation coefficients were also calculated for the two breeds studied to show the degree of relationship between RT and RR.

The Pearson's coefficients of correlation between the RT and RR of the White Fulani and the Sokoto Gudali were 0.73 and 0.87 respectively.

The rectal temperature and respiratory rates both showed distinct diurnal fluctuations, with the minimum values obtained at 08:00h while the maximum values were obtained at 15:00h, except for the respiratory rate of the Sokoto Gudali which peaked at 18:00h. Similar results were obtained on Holstein – Friesian cows[5] and on Friesian and Brown Swiss cows[6] in which there were also diurnal variations. The low respiratory rates observed in the present study appear to indicate a good level of adaptability of these indigenous breeds to the environment.

This study shows that ambient temperature changes affect rectal temperature, as revealed by distinct diurnal rhythm with peak values observed in the afternoon. This agrees with the observations of[7, 8, 5,] who showed that environmental temperature increased the rectal temperature. It was also observed in the present study that there was no difference, due to age, in the rectal temperature of the White Fulani cattle.

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AEROBIC BACTERIA ISOLATED FROM DEAD-IN-SHELL CHICKEN EMBRYOS IN KADUNA STATE, NIGERIA

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The poultry industry in Nigeria contributes a great deal to the Nigerian economy. Hatchery losses associated with embryonic mortality negatively affect the poultry industry resulting in low hatchability. Death In the shell of chicken embryos constitutes one of the major factors accounting for this low hatchability of incubated eggs[1,2]. This phenomenon is a common occurrence in Nigerian hatcheries which results in great and untold losses[3]. There is also paucity of information on the etiology of dead-in-shell chicken embryos in Nigeria.

In Anambra State of Nigeria alone, the hatcheries found here recorded registered percentage of dead-in-shell of 14.36% and 17.60% for eggs incubated in two farms investigated in 1985[4]. Other works where Falade isolated *E. coli* from such embryos belonging to hatcheries in Oyo State, Nigeria were done in 1976[5], and in 1983 by Akinyemi[3].

This study was therefore carried out to investigate the aetiology by the isolation and identification of bacteria from dead-in-shell chicken embryos with the aim of improving the poultry industry in Nigeria (following approved standards of poultry practice as stipulated in Haynes and Smith[6]) to meet up with increasing protein demand as the population increases.

The study area is Kaduna State which is located in the North Western region of Nigeria. At the study time (April, 20th – September, 13th; 2006), only four major

Table I: Distribution of isolates by sampling periods and the hatcheries

Source of Samples	18th day of incubation		21st day of incubation		Total no of	
	No of Dead-in-shell Embryos	Total No. Of isolates	No of eggs that failed to hatch	Total No. Of isolates from eggs	Eggs sampled	Isolates from samples
HatcheryA	70	8	130	18	200	26
HatcheryB	60	9	100	22	160	31
HatcheryC	54	5	80	15	134	20
HatcheryD	46	13	60	23	106	36
Total	230	35	370	78	600	113

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Table II: Bacterial Isolates and their distribution by isolation periods

Bacteria	Bacterial isolates on 18th day of incubation	Bacterial isolates on day of hatching	Total isolates
Escherichia coli	21	41	62
Proteus sp	5	16	21
Pseudomonas sp	0	6	6
Staph. aureus	5	6	11
Staph. sp	1	7	8
Micrococcus sp	3	2	5
Total	**35**	**78**	**113**

functional commercial hatcheries were present in this area and the sampling of unhatched eggs was done from the identified hatcheries. The sample size was determined by availability and selection (random selection without replacement) of eggs was based on intactness of the egg shells. The hatcheries were visited on a weekly basis depending on activities in the hatcheries. Eggs that failed to hatch were collected in containers from the hatcheries and taken to the laboratory with minimum delay. The egg shells were washed and disinfected properly to avoid surface and extraneous contamination. The contents of these eggs were cultured on bacteriological solid media, identification of growths and characterization were done following standard procedures stated in Cowan[7] and Cheesbrough[8].

Results

Of all the 600 dead-in-shell chicken embryos investigated in this study (table 1), a total of 113 Gram +ve and Gram –ve bacterial isolates were recovered. Table II shows the frequency of bacterial isolation in the four hatcheries and at the two sampling periods during incubation. Thirty five (35%) isolates were obtained from dead-in-shell embryos collected from the hatcheries on the 18th day of incubation. The remaining 78 (69%) isolates came from samples collected on the day of hatching. Of the 113 isolates, 89 were Gram negative bacteria comprising of 62 *Escherichia coli,* 21 *Proteus sp* and 6 *Pseudomonas spp.* The remaining 24 isolates were Gram positive bacteria made up of 11 *Staphylococcus aureus,* 8 other *Staphylococcus spp* and 5 *Micrococcus spp. Corynebacterium spp* (2 isolates) and *Bacillus spp* (1 isolate) were also obtained but at a very low frequency. On the 18th day of incubation, there were a total of 35 isolates out of which 21 were *E. coli* making the highest frequency while *Pseudomonas spp.* was lowest with no isolate at all. On day 21, *E. coli* was still highest with 41 isolates while *Micrococcus spp.* were 2, making the lowest frequency.

Discussion

Of all the bacteria isolated, the predominant species was *E. coli* (54%) followed by *Proteus spp* (18.6%). *Staphylococcus aureus* and other *Staphylococcus spp* together made up 16.8%. These findings contrast with those

of Bruce and Johnson[9] and Orajaka and Mohan[4] where Bruce and Johnson in a similar work found that *Micrococcus spp.* and *Enterobacteriae* represented 34.6% and 31.5% respectively; while *Staph. aureus* gave 9.2%. Orajaka and Mohan also in their study found *E. coli* to comprise of 25% and *Staph. aureus* 27.18% of the bacterial isolates. Laboratory tests carried out on the strains of isolated *Staph. aureus* showed the production of acid in mannitol both aerobically and anaerobically; also, delta and alpha hemolysis was produced and these findings agreed with those of Devriese and Oeding[10] for the strains of *Staph. aureus* isolated from poultry. It is significant that none of the isolates produced beta hemolysis, which is in agreement with the results obtained by other workers[11, 12].

Hatchery D had the lowest number of samples taken and yet recorded the highest number of isolates which meant the highest frequency of isolation. This result corresponded with the highest average percentage losses of 13%/week recorded in the preliminary investigation carried out. Hatchery A recorded the lowest frequency of isolation which also corresponded with the least average percentage losses of 8%/ week recorded by the hatcheries and revealed at the preliminary investigations. These facts support the idea that the bacteria isolated might have contributed to the death in the shell of the embryos especially that routine practices showed conformity with approved standards practiced[5] thus eliminating other causes of death in the shell other than microorganisms.

It could be inferred from the results that infection of the embryo can take place at any time within the incubation period. Thus appropriate hatchery sanitation is recommended. This involves setting up of clean eggs, personnel maintaining a high level of hygiene, the use of clean incubators, etc. This will reduce dead-in-shell due to bacteria.

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