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Assistant Prof., Nutrition - Ruminants, Nutritive Value, Utilization of Feeds
Nilüfer SABUNCUOĞLU ÇOBAN,
Dep. Animal Science and Production, Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**
Associate Prof., Ph.D**.** Animal Hygiene, Physiology, Animal Welfare
Osman Erganiş,
Dep. Microbiology, Facult. Vet. Med., Selcuk University, Konya, **TURKEY**
Ph.D., Prof., Food Safety, Physiology and Functional Biology of Systems
Ömer ÇOBAN,
Dep. Animal Science and Production, Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**
Associate Prof., Ph.D**.** Nutrition - Ruminants
Paola Roncada
Associate Professor – Veterinary Pharmacology and Toxicology, Department of Veterinary Medical Sciences, Faculty of Veterinary Medicine, University of Bologna, **ITALY**
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Saeid Chekani Azar,
Dep. Anim. Sci., Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**
Dep. Anim. Sci., Islamic Azad University (I.A.U.), Shabestar, **IRAN**
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Dep. Immunology, Faculty of medical Sciences, Juntendo University, **JAPAN**
Shahin Eghbal-Saeid,
Dep. Anim. Sci., I.A.U., Khorasgan (Isfahan), **IRAN**
Assiociate Prof., Ph.D., Animal Genetics and Breeding
Tohid Vahdatpour,
Dep. Physiology, Facult. Vet. Med., I.A.U., Shabestar, **IRAN**
Ph.D., Physiology and Functional Biology of Systems
Vassilis Papatsiros
Dep. Medicine (Porcine Medicine), Faculty of Veterinary Medicine, University of Thessaly, Trikalon str 224, GR 43100, **GREECE**
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Dep. Anim. Sci., Islamic Azad University (I.A.U.), Maragheh, **IRAN**
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Yousef Mehmannavaz
Dep. Anim. Sci., I.A.U., Maragheh, **IRAN**
Assistant Prof., Ph.D., Animal Genetics and Breeding
Zohreh Yousefi
Faculty of Biological Sciences, Shahid Beheshti University, Tehran, **IRAN**
Biology, Botanical Biosystematic (MSc), Plant Genetic (PhD student)

Deputy Section Editors (DSE, Reviewers)

Arda Yildirim
Department of Animal Science, Faculty of Agriculture, Gaziosmanpasa University, 60240 Tokatö **TURKEY**
Ph.D. (Assistant Professor), Animal Science, Nutrition-non Ruminants, Breeding, Nutritive Value, Utilization of Feeds
Behzad Shokati
Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh, **IRAN**
Agriculture: Environment, Nutritive value and utilization of feeds
FARHAD AHMADI
Dep. Anim. Sci., I.A.U., Shabestar, **IRAN**
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Ferdaus Mohd. Altaf Hossain
Sylhet Agricultural University, Bangladesh; not shah Jalal University of Science & Technology, **BANGLADESH**
D.V.M, Microbiology, Immunology, Poultry Science, and Public Health
Ibrahim Bushara Mohammed Ibrahim
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Animal Science, Nutrition-non Ruminants, Nutritive Value, Utilization of Feeds
Mutaz Saeed Babiker Mahmoud
Dep. Poult. Prod., Facult. Anim. Prod., University of Gezira, **SUDAN**.
Murtada Babiker Mohamed Elemam
Department of Animal Production, Faculty of Agriculture and Natural Resources, University of Kassala, P.O. Box 12, New Halfa, **SUDAN**
Ph.D. Nutrition - Ruminants (Ruminant Nutrition, Microbes and Physiology)
Navid Hosseini Mansoub,
Dep. Anim. Sci., I.A.U., Maragheh, **IRAN**
DVM, Pathology
Raga Mohamed Elzaki Ali
Dep. Rural Economics and Development, Faculty of Animal production- Managil, University of Gezira, **SUDAN**
Ph.D. (Assistant Professor), Animal-feed interactions, Nutritive value and utilization of feeds
Shahin Hassanpour
Dep. Physiology, Facult. Vet. Med., I.A.U., Shabestar, **IRAN**
Physiology and Functional Biology of Systems
Terry Ansah
Ph.D. student, University for Development Studies-Ghana and Harper Adams University College, **UK**
Nutrition - Ruminants
Yadollah Bahrami,
Dep. Anim. Sci., I.A.U., Khorasgan (Isfahan), **IRAN**
Ph.D. Student, Nutrition - Non-Ruminants
Tarlan Farahvash
Dep. Anim. Sci., I.A.U., Khorasgan (Isfahan), **IRAN**
Tarbiat Modares University, Tehran, **IRAN**
Ph.D. Student, Animal Genetic and Breeding

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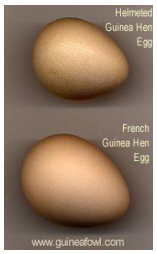
Research Title/ Field	Article (Abstract)	Download
A comparative study of local Ghanaian maize, imported yellow maize and two new quality protein maize (QPM) varieties – Etubi and Golden Jubilee – Effects on growth performance and carcass characteristics of pigs 	**Original Research, B41** **Salifu, A-R.S., Okai, D.B., Boateng, M. and Ewool, M.B.** ***Online J. Anim. Feed Res.*, 2(3): 218-223, 2012.** **ABSTRACT:** *The experiment was conducted to determine growth performance and carcass characteristics of growing-finishing pigs fed diets containing four different varieties of maize. Twenty individually- housed, Large White pigs (12 males and 8 females) with an average initial body weight of 13.3 kg were allotted to four dietary treatments labelled, Local Normal (LN), Imported Normal Yellow (INY), Golden Jubilee (GJ) and Etubi (ET) in a Completely Randomized Design (CRD). Each treatment was replicated five times, with a pig representing a replicate. Feed and water were offered ad-libitum and growth performance was monitored over the trial period (13-70kg liveweight). There were no significant effects of diets on ADFI and FCE but ADWG and feed cost per kg gain were influenced by the diets. The values were 0.64, 0.61, 0.56 and 0.60 kg and GH¢1.74, GH¢1.90, GH¢1.76 and GH¢1.75 for the LN, INY GJ and ET treatments respectively. The values for LN, GJ and ET were statistically similar (P>0.05). Values for carcass length, dressing percentage, shoulder, loin, belly, thigh, and bac kfat thickness were not statistically different (P>0.05) between the four dietary treatments. However, there were significant differences (P<0.05) in the values for heart, liver, spleen, full gastrointestinal tract (GIT) and the respiratory tract. The results indicated that using GJM and ETM varieties could be more economical and could lead to the production of leaner pork carcasses.* **Key words:** *Growth Performance, Carcass Characteristics, Golden Jubilee Maize, Etubi Maize, Pigs*	
In Vitro Ruminal Protein Degradability of Leaves From Three Tree Species Harvested at Two Cutting Intervals  *Gliricidia sepium*  *Leucaena leucocephala*  *Trichanthera gigantea*	**Original Research, B42** **Edwards A, Mlambo V, Lallo CHO, Garcia GW, Diptee M.** ***Online J. Anim. Feed Res.*, 2(3): 224-230, 2012.** **ABSTRACT:** *In vitro ruminal protein degradation characteristics of protein supplements represent an accurate measure of the quality of protein for ruminant animals. As such, crude protein disappearance of Gliricidia sepium, Leucaena leucocephala and Trichanthera gigantea leaves, which are potential sources of supplemental protein for ruminants, was determined using the ANKOM in vitro ruminal degradability technique. Dry matter (DM) and crude protein (CP) disappearance were measured after 0, 2, 4, 6, 12, 24, 36, 48 and 72 h of incubation. Degradation kinetics were described using the Ørskov and McDonald equation $y=a+b(1-e^{-cx})$. The degradable part of the insoluble DM fraction (b) was highest (P<0.05) in G. sepium leaves (27%) at the 12 week cutting interval. Effective dry matter degradability (EDMD) was highest (P < 0.05) in the leaves of G. sepium (74.9%) at the 12-week cutting interval. CP washing losses was highest (P < 0.05) in the leaves of L. leucocephala (46.8%) and lowest in T. gigantea leaves (16.3%) at the 6-week cutting interval. Crude protein disappearance was highest (P<0.05) in the leaves of G. sepium and lowest in T. gigantea leaves at both the 6 and 12-week cutting intervals after incubation at 48 h. It is concluded that in vitro ruminal protein degradability is more pronounced in the leaves of G. sepium and L. leucocephala. Approximately 50% of their protein is degraded in the rumen suggesting that they would be useful as sources of readily available nitrogen for rumen microbes challenged with low nitrogen, fibrous basal diets. Trichanthera gigantea leaves have higher levels of rumen undegradable protein suggesting that they can be used to supply by-pass protein for animal.* **Key words:** *In Vitro Rumen Degradability, Protein Quality, Effective Degradability, Harvesting Frequency, Tree Forages*	
Preliminary on–station study of growth performance of grower pigs on ensiled cassava pulp and dried cassava leaves 	**Original Research, B43** **Rhule SWA, Asiedu P, Ameleke GY, Baiden RY, Sottie ET, Otsyina HR** ***Online J. Anim. Feed Res.*, 2(3): 231-234, 2012.** **ABSTRACT:** *The performance of grower pigs on diets containing graded levels of cassava pulp, cassava peels and dried cassava leaves was studied. Twenty-four Large White grower pigs at an average initial live-weight of 20 kg were distributed over six diets by the completely randomized design. The pulp was preserved by ensiling in polyethylene bags for a period of three months before use. The pigs were group-fed once-daily for five weeks. The average daily gains (ADG) of the pigs were 0.27, 0.19, 0.28, 0.26, 0.15 and 0.20 kg live-weight gain/day on diets 1, 2, 3, 4, 5 and 6 respectively. The cost of feed were 0.16, 0.15, 0.15, 0.13, 0.12 and GH¢0.10 per kg of feed for diets 1, 2, 3, 4, 5, and 6 respectively. The corresponding economy of gain (EG) were 0.58, 0.74, 0.53, 0.49, 0.72 and GH¢0.49. The highest inclusion rate was 30% for the pulp and 20% for the leaves. The pigs were weighed weekly over a five week period. Whereas the ADG of the pigs in this study was best on diet 3 (25% pulp), the EG was best on the diets 4 (30% pulp) and 6 (20% cassava leaves).* **Key words:** *Ensiled Cassava Pulp, Dried Cassava Leaves, Large White Grower Pig, Average Daily Gain, Economic of Gain*	
Nutritive value of rice polish 	**Original Research, B44** **Hossain ME, Sultana S., Shahriar SMS, Khatun MM.** ***Online J. Anim. Feed Res.*, 2(3): 235-239, 2012.** **ABSTRACT:** *The present study was undertaken to observe the chemical composition of different types of rice polish available in different areas of Chittagong, Bangladesh. Twenty different types of rice polishes were collected from study areas. Chemical analyses of the samples were carried out in triplicate for moisture, dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extract (NFE), ether extract (EE) and total ash (TA) in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. Metabolizable energy (ME) was calculated mathematically for all samples by using standard formula. Results indicated that, there*	

	were no marked variations (P>0.05) in the moisture, DM and TA contents of the samples. However, ME, CP, CF, NFE and EE content significantly differed (P□0.01) from one sample to another. Moisture content varied from 4.0 to 11.4 g/100g, DM content varied from 88.6 to 96.0 g/100g, ME content varied from 1321.8 to 3086.9, CP content varied from 4.7 to 14.9 g/100g, CF content varied from 6.4 to 41.5 g/100g, EE content varied from 1.0 to 18.0 g/100g, NFE content varied from 25.1 to 52.9 g/100g and TA content varied from 7.1 to 17.6. It could therefore, be inferred that, the chemical composition rice polish currently available in the local market are widely variable. **Key words:** *Rice Polish, Moisture, Dry Matter, Crude Protein, Crude Fiber, Nitrogen Free Extract, Ether Extract and Total Ash*	
Substitution of lysine with mushroom (*Pleurotus cystidiosus*) in broiler chick's diet 	**Original Research, B45** **Ezeonyejiaku CD, Ebenebe CI, Okeke JJ, Obiakor MO, Ezenwelu CO.** ***Online J. Anim. Feed Res.*, 2(3): 240-243, 2012.** **ABSTRACT:** *Effect of inclusion of mushroom (Pleurotus cystidiosus) to substitute lysine in the diet of broiler chicks was investigated. The study lasted for a period of twelve weeks. Twenty four broiler chicks were subjected to two different dietary treatments (Diet I contained 0.22% of mushroom while Diet II contained 0.22% of synthetic Lysine and was used as control). The different treatments had four replicates of three birds each housed in a metabolic cage. Two parameters, mean weight gain and mean feed intake were recorded. Student t- test showed that there was no significant difference (P>0.05) in the mean weight gain for the chicks on the two treatments (DI-3550g and DII-3375g) and mean feed intake for the chicks on the two treatments (DI-502.5g and DII-420g). Consequently, the observed results showed that mushroom can be used to substitute lysine in the diet of broiler chicks.* **Key words:** *Mushroom, Lysine, Broiler Chicks, Amino-Acid*	
Evaluation of I_2 thermostable Newcastle disease vaccine on local chickens in selected districts of western Amhara 	**Original Research, B46** **Nega M, Moges F, Mazengia H, Zeleke G, Tamir S.** ***Online J. Anim. Feed Res.*, 2(3): 244-248, 2012.** **ABSTRACT:** *Evaluation of l2 thermostable Newcastle disease vaccine was conducted in three districts of four local chicken ecotypes using survey and sera analysis from 2010 to 2011. According to the survey result conducted on 160 chicken owners, the major chicken production constraint 77.5% of the area was disease and mortality of chickens by any cause from day old to adult chicken age was 44.6%, ranging from 39.9%, 45.3%, 45.8%, and 46.3% at Melohamusit, Mecha, Farta, and Tillili, respectively from which disease related mortality was 77%. Mortality of chickens due to disease outbreak was usually higher during the beginning of the rainy season, mainly in April (43.1%), May (38.8%) and June (63.8) as first, second and third priority months, respectively and there is significant deference in disease occurrence among seasons. The overall seroprevalence of Newcastle disease in village chickens using Hemagglutination inhibition test (≥1:16) was 55.8%. However, the antibody titer response to I2 thermostable vaccine was 90.4% ranging from 83.8%, 90.9%, 91.7%, 95.1% in Mecha, Tillili, Farta and Melohamusit, respectively after one vaccination and 93% ranging from 90.9%, 93.3%, 93.8%, 96%, in Mecha, Melohamusit, Tillili and Farta, respectively after booster dose vaccination. There was no significant difference in antibody titer detected between local chicken ecotypes and/ or districts before and after vaccination. However, there was significant difference in antibody titer after 1st (P =0.000) and booster dose (P =0.000) vaccination. A quick survey conducted after the last vaccination showed that mortality of chickens became 8.2% which is reduced by 82% than the mortality before vaccination. In conclusion this vaccine was found very appropriate and effective in reducing village chicken mortality and morbidity, so controlling of Newcastle disease using I2 thermostable vaccine could be a key to the development of village chicken production.* **Key words:** *Hemagglutination, I_2 thermostable vaccine, Newcastle disease, Village chickens*	
Some behavioral traits of red neck ostrich under captive conditions 	**Original Research, B47** **Mohammed Ahmed FA, Mohammed Salih RR.** ***Online J. Anim. Feed Res.*, 2(3): 249-252, 2012.** **ABSTRACT:** *The present study has been conducted to observe some behavioral traits of ostrich under captive conditions. The observations have been carried during the period 14 June to 24 June, 2005, for 8 equal time period, extending for 24 hours from 0600 p.m hour to 0600 p.m hour next day. The bird flack consisted of two adult males and adult female, kept in the Collage farm, in a cage joined to a fence to allow for free movement. The recorded behavioral activities included: standing in the sun, standing in shade, laying in the shade, laying in the sun, staying in the cage, movement and sitting on the knees, feeding, drinking, quarrel, urination, defecation, ritual display, courtship, and preening. It was noticed that the most time consuming activities were standing in the sun, standing in the shade, laying in the shade, and movement. The longest period of the time budget was taken in laying in shade (250.3 min.). The shortest fraction of the time budget was spent in courtship maneuvers (3.25 min.). The main target of the study was to provide ostrich breeders with useful information for better management.* **Key words:** *Behaviour, Ostrich, Captivity Condition, Birds*	
Hatchability of guinea fowls eggs and performance of keets under the traditional extensive system in Tolon-Kumbungu district of Ghana	**Original Research, B48** **Naandam J, Issah GB,** ***Online J. Anim. Feed Res.*, 2(3): 253-257, 2012.** **ABSTRACT:** *A study was carried out to examine the hatchability of guinea fowls eggs and performance of keets under the traditional extensive system. A short questionnaire to ascertain production scope and management practices were administered to a total of ten farmers; five farmers from each of two communities, using purposive sampling. In order to establish some actual production indices, data was collected from the sampled farmers on mean number of eggs incubated, mean weight of eggs incubated, mean number of eggs hatched, percentage hatchability of eggs, mean weekly numbers of keets, mean weekly weight gain of keets, total weight gain of keets and mortality rate of*	



keets. Data were analyzed using Genstats Discovery (3rd edition) and SPSS version 17. The main breeds of guinea fowls kept by farmers were the pearl and the lavender. The methods of identifying fertile eggs by farmers were by the use of size and texture of eggs. Majority of the farmers (80%) fed their guinea fowls with maize, while (20%) fed them with millet before egg laying, but during egg lay 80% of the farmers fed their guinea fowls with millet for the reason that it increased egg production. For the production indices, there were significant differences (P<0.001) in mean weekly numbers of keets and mean weekly weight gain of keets for the study period. A much lower significant difference (P<0.05) was observed for the total weight gain of keets, possibly because weight gain through growth over stripped the weight losses through mortality. Mortality rate of keets was high ranging between 61-69% within the two communities, though these did not significantly differ from each other. Mean number of eggs incubated was 18.4 for Nafaring community and 25.4 for Cheyohi community. Similarly the mean weight of eggs incubated, total weight of eggs incubated, number of eggs hatched and percentage hatchability (%) were 31.4g and 31.8g, 577.8g and 807.7g, 13.4 and 18.6, 72.8% and 73.6%, respectively. There were significant differences in performance indices across the weeks but not between the two communities.

Key words: *Communities, Hatchability, Keet Performance Traditional Extensive System, Mortality*

Degradation characteristics of some Sudanese grasses and gas production techniques

Original Research, B49

Idris A.O., Kijora C., Salih A.M., Bushara I., Elbukhary H.A.A.

***Online J. Anim. Feed Res.*, 2(3): 258-263, 2012.**

ABSTRACT: *Eighteen plant species, three ingredients, and six diets were studied for their degradation characteristics, using gas production techniques. The palatable grasses were selected during the rainy season from the range land of Kordofan, Sudan. The ingredients were Roselle seeds, Sorghum grain and Groundnut cake. The samples were incubated for 4, 8, 12, 24, 48, 72 and 96 h, using rumen inoculum of three of the sheep used for the nylon bag. The results showed a large variation between the different plant species in the gas volume. The potential gas volume reflected the presence of anti-nutritional factors. Gas production from the ingredients indicated that sorghum grain recorded the highest gas production volume. The gas production at different time intervals showed increased degradability in the grasses, diets and the ingredients. Eragrostis tremula could be used as reference forage in evaluating the organic matter digestibility and energy density of grasses and Farsefia longisiliqua as a reference for crude protein.*

Key words: *In vitro, Gas production, Grasses degradability, Rangeland of Kordofan, Sudan*

Inventory and development perspective of milk production in Saharan area: the case of the Ghardaïa region (Algeria)

Original Research, B50

Bensaha H, Mayouf R, Bensaha L.

***Online J. Anim. Feed Res.*, 2(3): 264-269, 2012.**

ABSTRACT: *The National Fund for the Development of Agricultural Investments (FNDIA) supports various actions, including the dairy industry (mini-dairy, production and birth bonuses, milk collection, processing and artificial insemination). At the level of the Ghardaïa region, like the other Saharan regions, FNDIA helped initiate the development of livestock and thereby contributed to the increase in the number of head of cattle. The establishments of nurseries and of specialized dairy barns have created a dynamic in the dairy cattle farming and have positive impacts on the local market, namely an increase in the production of milk. According to the Directorate of Agricultural Services (DSA) of the Wilaya of Ghardaia (2010), the number of imported dairy cattle between 1995 and 2010 rose from 177 to 1688 dairy cows owned by the private sector. 13 400 liters of milk are collected daily by dairies and milk collection points. In this context, the objective of this research is to develop an inventory of the dairy industry in Ghardaia and identify its strengths and weaknesses in order to propose solutions to ensure its sustainability and thus provide guidance to the strong investment by government.*

Key words: *Agricultural Development, Dairy Cattle, Ghardaïa, Milk Production, Saharan Region*

Residue depletion of sulphadiazine and trimethoprim in pigs and broilers after oral administration

Selected by OJAFR editors as Hot Paper in terms of careful work, write and submission

Original Research, B51

Roncada P., Tomasi L., Sori F., Zaghini A., Zaccaroni A., Ferrara D.

***Online J. Anim. Feed Res.*, 2(3): 270-276, 2012.**

ABSTRACT: *The residual behaviour of a sulphadiazine (SDZ) and trimethoprim (TMP) combination was studied in fourteen pigs and twenty-eight broilers. The drug combination was added in the amount of 700 mg kg^{-1} (SDZ) and 140 mg kg^{-1} (TMP) to pig and 300 mg kg^{-1} (SDZ) and 60 mg kg^{-1} (TMP) to broiler feed, respectively. The medicated feeds were supplied for 5 consecutive days. The tissue SDZ/TMP concentrations were measured by a HPLC method. To ensure safe residue levels in all target tissues, withdrawal time of 8.6 days and 6.0 days should be applied to pigs and broilers, respectively, treated with SDZ and TMP in feed.*

Key words: *Sulphadiazine; Trimethoprim; Pigs; Broilers; Residues; Withdrawal Time; Veterinary Drugs*

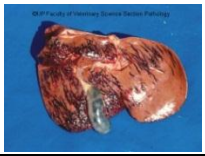
Marketting situations of livestock feeds in Welmera and Dendi Wereda of west Shoa zone, Ethiopia

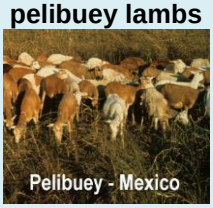
Original Research, B52

Mesfin R., Tesfaye A.

***Online J. Anim. Feed Res.*, 2(3): 277-282, 2012.**

ABSTRACT: *The paper explains the status of livestock feed resources and market situations in Welmera and Dendi weredas of West Shoa Zone, Ethiopia. The objective of the survey was to assess the potentials and constraints of feed resources and related marketing practices and suggest appropriate intervention options to overcome the constraints. Majority (76%) of the interviewed farmers have faced shortage of livestock feeds. The diminishing trend of grazing land from time to time, roughage, concentrate feeds are the factors contributing to feed shortage. Moreover, the increasing trend in selling price of hay and concentrate feeds aggravates more to the problem. This situation is limiting livestock productive in the highlands of Ethiopia. Under this condition, farmers*

	purchase feeds to both local and crossbred animals. The purchased feeds include: hay, straw, grazing area, oilseed cakes, wheat bran and wet grass. Among these, the grazing area purchased takes the highest (52%) proportion. Farmers and traders participate in purchasing of livestock feeds. The proportion of farmers that purchase feeds is higher (30%) than that of the traders (1%). To alleviate the problems related to shortage of livestock feeds and decline of animal production and productivity, rearing of improved crossbred dairy cattle under intensive management and forage/fodder development and feeds conservation schemes should be promoted in a wider scale. Considering the ever-increasing price of feeds, there is a need to shift from purchased commercial feeds to the use of farm produced feed resources. **Key words:** *Farmers, Grazing Land, Roughage, Concentrate Improved Forage*	
Addition of protein sources for calves supplemented with high moisture sorghum grain silage grazing low-quality pastures 	**Original Research, B53** **Rovira, P.** ***Online J. Anim. Feed Res.*, 2(3): 283-287, 2012.** **ABSTRACT:** *Three experiments were conducted to determine the effect of protein addition to high moisture sorghum grain silage (HMS) daily supplemented to calves at a rate of 1% of body weight (BW) grazing low-quality pastures. In exp. 1 addition of sunflower expeller or a protein ration to increase crude protein (CP) of HMS from 7.1% to 12% increased average daily gain 56% compared with calves fed only HMS (0.39 and 0.25 kg/a/d, respectively). Calves supplemented with protein sources were more efficient than calves supplemented only with HMS as feed conversion numerically decreased from 6.0 (HMS) to 4.5 (HMS + sunflower expeller) and 4.1 (HMS + protein ration). In exp. 2 CP of HMS (9.1%) was increased to 15.5% by adding sunflower expeller, urea or combination of both. Protein supplementation increased ADG and final BW (0.20 kg/a/d and 196 kg) compared with only HMS (0.03 kg/a/d and 176 kg). Protein source had no effect on animal performance. In exp. 3 CP concentrations in the supplement had a significant effect on ADG when increased from 8.9 to 16.1% (0.32 and 0.50 kg/a/d). Performance of calves fed either 16.1% or 20.8% CP supplements did not differ possibly because energy was becoming the limiting factor at the highest CP concentration level. Rib eye area and fat thickness were not affected by treatment although supplemented calves registered 7% and 10% greater values in those variables, respectively, than un-supplemented animals at the end of the experiment. The addition of protein sources to HMS increased performance of calves grazing low-quality pastures.* **Key words:** *Calves, Pastures, Sorghum Silage, Protein Addition, Supplementation*	
Nutritive value of sawdust	**Original Research, B54** **Hossain ME, Rahman MJ and Islam KMF.** ***Online J. Anim. Feed Res.*, 2(3): 288-291, 2012.** **ABSTRACT:** *The present study was undertaken to observe the chemical composition of different types of sawdust available in the urban and peri-urban areas of Chittagong, Bangladesh. Twenty different types of sawdust from different plants were collected from study areas. Chemical analyses of the samples were carried out in triplicate for moisture, dry matter (DM), metabolizable energy (ME), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and total ash in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. Results indicated that, there were no variations (P□0.05) in the DM, EE and TA contents of the sawdust samples. However, ME, CP, CF and NFE content differed (P□0.01) significantly from one sample to another. DM content varied from 91.6 to 97.4 g/100g, ME content varied from 535.9 to 1756.7 kcal/kg, CP content varied from 1.8 to 3.5 g/100g, CF content varied from 39.5 to 74.0 g/100g and NFE content varied from 12.5 to 47.1 g/100g. It could therefore, be inferred that, sawdust currently available in the local market widely varies in chemical composition.* **Key words:** *Sawdust, Dry Matter, Metabolizable Energy, Crude Protein, Crude Fiber, Nitrogen Free Extracts, Ether Extracts, Total Ash*	
Strain effect on some productive and reproductive performance traits of local improved Egyptian and Canadian chickens 	**Original Research, B55** **Taha A.E., Abd EL-Ghany F.A., Sharaf M.M.** ***Online J. Anim. Feed Res.*, 2(3): 292-300, 2012.** **ABSTRACT:** *This experiment was conducted to evaluate the effect of strain on some productive as well as some reproductive traits of local improved dual purpose three Canadian strains (Shaver A, B and C) and two Egyptian chicken strains (Salam and Mandarah). Results revealed that strain effect was evident for shaver C strain for (body weight at sexual maturity, body weight at 90 days of egg production, 42 and 65 weeks of age), also strain effect was evident for shaver C strain for feed consumption (at sexual maturity, 90 days of egg production, 42 weeks and 65 weeks of age) and (egg weight at 90 days of egg production, 42 and 65 weeks of age). While strain effect for fertility, hatchability and scientific hatchability, age at sexual maturity, Egg number at first 90 days of egg production and egg number at 42 and 65 weeks of age were recorded for Egyptian chickens. Moreover, negative correlation estimates were observed between age at sexual maturity and egg number at different periods as well as positive correlation between body weight at 8 weeks of age and most of productive traits that of high great benefits to select for economic traits in chickens at earlier age.* **Key words:** *Strain, Egg Parameters, Egypt, Fertility, Hatchability, Correlation*	
Evaluation of Indirect ELISA in Diagnosis of Natural Ovine Cysticerciosis and Haemonchosis 	**Original Research, B56** **Sultan K., Desouky, A.Y., Elbahy, N.M. and Elsiefy, M.A.** ***Online J. Anim. Feed Res.*, 2(3): 301-302, 2012.** **ABSTRACT:** *This study aimed to evaluate the sensitivity and specificity of indirect ELISA in diagnosis of natural infection of sheep with Cysticercus tenuicollis and Haemonchus contortus the most prevalent parasitic helminths in Egyptian sheep. By using non-purified crude antigens derived from the whole cyst of C .tenuicollis and adults H.contortus in the indirect ELISA assay; the results showed that both antigens sensitivity were 90%, 87.5% and the specificity were 60% and 75% respectively. These data proves the suitability of ELISA in diagnosis of such infections in living animals and the necessitation of using purified antigens rather than non-purified to increase the accuracy*	

	of the assay. **Key words:** *ELISA, Ovine, Cysticercus, Haemonchus*	
Growth of poultry chicks fed on formulated feed containing silk worm pupae meal as protein supplement and commercial diet	**Original Research, B57** **Dutta A, Dutta S, Kumari S.** ***Online J. Anim. Feed Res.*, 2(3): 303-307, 2012.** **ABSTRACT:** *Waste silkworm pupae (SWP) generate vast resources of nutrients for livestock and poultry. In the present investigation, three days old chicks of RIR strain were allocated to five dietary treatments of silk worm pupae meal. The energy budget was prepared from calculated proximate analysis and growth performance of broiler chicks fed with different percentages of silk worm pupae. The result showed that the silkworm powder meal (SWPM) is the cheapest and has potential to replace the costly and contaminated fish meal, as the protein source, used in poultry industry.* **Key words:** *Poultry; Fish Meal; Silkworm Pupae Meal; Proximate Analysis; Growth Performance; Energy Budget*	
Effect of tartaric acid addition on rumen fermentation, methane production and digestibility in different diets containing wheat straw *in vitro* 	**Original Research, B58** **Sirohi S.K., Pandey P., Goel N., Mohini M., Kundu S.S.** ***Online J. Anim. Feed Res.*, 2(3): 308-313, 2012.** **ABSTRACT:** *The aim of the current study was to evaluate the effect of tartaric acid addition in diets on in vitro methanogenesis and rumen fermentation. Different levels of tartaric acid (5, 10, and 15 ppm) were tested for their effect on methanogenesis, rumen fermentation and digestibility in three wheat straw containing diets i.e. Low fiber diet (LFD, 40R:60C), medium fiber diet (MFD, 50R:50C) and high fiber diet (HFD, 60R:40C). Evaluation of tartaric acid was carried out using in vitro gas production technique. Methane production and individual fatty acids were estimated by Gas Chromatography. Results of different levels of tartaric acid on in vitro methanogenesis indicated that the maximum methane reduction (22.60% in term of mM/gDM) was observed in LFD at the supplementation dosage of 15 mM and a similar trend was seen, when methane was expressed in ml/gDM. Non-significant (P≤0.05) effect of tartaric acid addition on in vitro dry matter digestibility (IVDMD) was observed in almost cases. Protozoal population decreased with increasing concentration of tartaric acid and maximum reduction (54.64%) was in the MFD. Acetate to propionate ratio was decreased in tartaric acid supplemented diets which reflects increase in propionic acid production in comparison to control diet. Microbial biomass yield also increased due to the addition of tartaric acid in most of the diets.* **Key words:** *Tartaric acid; Rumen fermentation; IVDMD, Microbial biomass; Methane production*	
Biometry and testicular growth influenced by nutrition on prepubertal pelibuey lambs  **Selected by OJAFR editors as Hot Paper in terms of careful work, write and submission**	**Original Research, B59** **Martinez JM, Dominguez B, Barrientos M, Canseco R, Ortega E, Lamothe C.** ***Online J. Anim. Feed Res.*, 2(3): 314-321, 2012.** **ABSTRACT:** *The growth and testicular development was studied in 48 Pelibuey male lambs 76.6±3.0 days of age and 12.7±1.9 kg body weight (BW), two groups were designed (n=24). 1: Intensive rotational grassing (IRG), 2: Intensive rotational grassing plus nutritional supplement (IRGS). BW was recorded every 15 days from 75 days of age to the onset of puberty. The animals grazed on Panicum maximum. IRGS received a concentrate with 15% of protein. The testicular biometry included scrotal circumference (SC) and testicular volume (TV). Blood samples were collected each 15 days from 90 to 190 days of age for evaluate the testosterone concentrations. BW, SC and TV at histological puberty was higher in IRGS than IRG; 22.5±1.5 vs. 16.06±1.5 kg, 22.0±1.0 vs. 12.2±1.5 cm, 60.5±1.7 vs. 12±3.5 cm^3 respectively (P<0.05) with an average age for the two groups of 162±7.0 days. The correlation coefficient (R) was higher (P<0.05) for SC vs BW than age vs. BW (0.884 vs. 0.816) and the TV vs. BW than TV vs. age (0.849 vs. 0.777) in the IRGS; the IRG showed lower R for the same comparisons (P<0.05). Seminiferous tubules showed lumen by day 142, spermatids and spermatozoids by day 171 for IRGS, meanwhile in the IRG only showed gonocytes and Sertoli cells. Testosterone concentrations reached a peak (2.5 ng/ml) at 168 days of age for the IRGS meanwhile the IRG showed lower levels than 0.05 ng/ml. Testicular development and testosterone concentrations depends more on BW than age; and they are modified by the nutritional management in prepuberal male lambs.* **Key words:** *Testis Development, Puberty, Nutrition, Lambs*	
Preliminary investigation of aflatoxins in dietary ration of dairy cows in Khartoum state, Sudan  	**Original Research, B60** **Elteib W.O.M., El Zubeir I.E.M., Fadel Elseed A.M.A., Mohamed A.A.** ***Online J. Anim. Feed Res.*, 2(3): 322-327, 2012.** **ABSTRACT:** *This is a preliminary investigation of the incidence and levels of aflatoxins in dairy cow ration in Khartoum North locality using HPLC. The survey was based on three level of groundnut cakes concentration (low=16-18, medium=19-24 and high=25-32%). The data indicated that 2 out of 18 samples examined were contaminated with aflatoxins B1 (0.013 and 0.014 ppb), these values were below the maximum acceptable limit for dairy cows feeds (20 ppb) as was stated by FAO (1997). However further examination of 2 samples of groundnut cakes from the farms showing the positive sample, revealed 108.3 and 18.4 ppb for B1 and 71.6 and 12.4 ppb for B2, respectively. The study also suggested a relationship between the levels of groundnut cakes level in the feed ration of the dairy cows and the contamination by aflatoxins, as these positive samples were from feed ration of high level of groundnut cakes concentration. The positive samples were from dairy farms that mixed their own ration using a traditional mill. The study also showed the absence of G1, G2 and B2 in dairy cows feeding in Khartoum North locality. From this study it was concluded that ration formulation with different feedstuff could minimized the aflatoxins health risk for dairy animals, however further research is needed in this field.* **Key words:** *Aflatoxins, Groundnuts Cakes, Dairy Cows, Contamination*	

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A COMPARATIVE STUDY OF LOCAL GHANAIAN MAIZE, IMPORTED YELLOW MAIZE AND TWO NEW QUALITY PROTEIN MAIZE (QPM) VARIETIES – ETUBI AND GOLDEN JUBILEE – EFFECTS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF PIGS

A-R.S. SALIFU[1], D.B. OKAI[1], M. BOATENG[1]*, M.B. EWOOL[2]

[1]Department of Animal Science, Faculty of Agriculture, College of Agriculture & Natural Resources, Kwame Nkrumah University of Science & Technology, Kumasi-Ghana
[2]CSIR-Crops Research Institute, Kumasi-Ghana

*Email: michaelboateng@knust.edu.gh

ABSTRACT: *The experiment was conducted to determine growth performance and carcass characteristics of growing-finishing pigs fed diets containing four different varieties of maize. Twenty individually- housed, Large White pigs (12 males and 8 females) with an average initial body weight of 13.3 kg were allotted to four dietary treatments labelled, Local Normal (LN), Imported Normal Yellow (INY), Golden Jubilee (GJ) and Etubi (ET) in a Completely Randomized Design (CRD). Each treatment was replicated five times, with a pig representing a replicate. Feed and water were offered ad-libitum and growth performance was monitored over the trial period (13-70kg liveweight). There were no significant effects of diets on ADFI and FCE but ADWG and feed cost per kg gain were influenced by the diets. The values were 0.64, 0.61, 0.56 and 0.60 kg and GH¢1.74, GH¢1.90, GH¢1.76 and GH¢1.75 for the LN, INY GJ and ET treatments respectively. The values for LN, GJ and ET were statistically similar (P>0.05). Values for carcass length, dressing percentage, shoulder, loin, belly, thigh, and bac kfat thickness were not statistically different (P>0.05) between the four dietary treatments. However, there were significant differences (P<0.05) in the values for heart, liver, spleen, full gastrointestinal tract (GIT) and the respiratory tract. The results indicated that using GJM and ETM varieties could be more economical and could lead to the production of leaner pork carcasses.*

ORIGINAL ARTICLE

Key words: *Growth Performance, Carcass Characteristics, Golden Jubilee Maize, Etubi Maize, Pigs*

INTRODUCTION

Maize is an indispensable cereal grain in the diets of monogastric animals and forms about 50-60% of such diets (Osei et al., 1999 and Okai and Boateng, 2007). Its use is the result of a combination of desirable nutritional characteristics. It is high in energy, low in fibre, palatable and easily digested (NRC, 1988). The normal maize varieties used in Ghana and elsewhere have two major limitations, namely, low protein (9-10%) and deficiency of some essential amino acids particularly lysine (0.23%) and tryptophan (0.06%) which do not meet the nutrient requirement of monogastric (Beeson et al., 1996). Maize-based diets are often supplemented with soyabean and fish meals to meet the requirements of the monogastric animal. Soybean meal and fish meal may be limited in supply in Ghana and the bulk of these are imported thus making fish meal and soybean meal very expensive at certain times of the year.

The quest of scientists for finding conventional ways of improving existing maize varieties with a better balance of essential amino acids led to the discovery of Opaque-2 and floury-2 and later, the development of QPM varieties. These varieties have nutritional superiority over the normal maize varieties (NRC, 1988) and elsewhere they have been evaluated with rats (Mertz et al., 1964, Nelson et al., 1965; Bressani et al., 1968; Maner et al., 1971, Maffia et al., 1976 and Serna-Saldivar et al., 1991). In growth trials, Sproule et al. (1988) and Sullivan et al., (1989) reported that QPM has a higher nutritive value than normal maize when fed in low protein diets containing the same level of supplemental protein. In Ghana, similar studies were carried on Obatanpa (an open pollinated QPM variety) upon its release. For example, Osei et al. (1999) reported that pigs on the QPM diets grew 2.36 times faster than those on the normal maize. Two new QPM varieties have recently been developed by the Crop Research Institute of Ghana based in Kumasi, namely; Golden Jubilee maize (GJ) and Etubi maize (ET). The Golden Jubilee is a yellow, dented and open-pollinated QPM variety with potential yields of 5 tons/ha and matures in 105 to 110 days whiles "Etubi" on the other hand, is a white flint and dented QPM hybrid with potential yield of 6.5 tons/ha and

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having the same months of maturity. In spite of these encouraging yield figures and positive agronomic attributes as well as the perceived nutritional value, there is a dearth of information on the responses of pigs to these new varieties. Therefore, this study therefore seeks to compare the effects of Local normal maize, imported normal yellow, GJM and ETM- based diets on growth performance and carcass characteristics of pigs.

MATERIALS AND METHODS

Study Area and Duration of Experiment

The study was conducted at the Livestock Section of the Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and the feeding trial lasted for 17 weeks.

Sources of feed ingredients

The Local Normal (LN), Etubi (ET) and Golden Jubilee (GJ) maize varieties were provided by Alpha Seeds Enterprise, Kumasi while the Imported Normal Yellow (INY) maize and other ingredients were bought from open markets in the Kumasi Metropolis.

Experimental pigs and design of the experiment

Twenty Large White starter pigs (12 males and 8 females) with an average age of 11 weeks obtained from the Livestock Section of the Department of Animal Science, KNUST were used in the experiment. The pigs were randomly allotted to four dietary treatments; namely LN, INY, GJ and ET diets on the basis of sex, litter origin, age and weight. A Completely Randomized Design, with 5 replicates per treatment was used. The compositions of the four isonitrogenous and isocaloric diets are shown in Table 1.

Table 1 - Percentage composition of the experimental diets

Ingredient	LN	INY	GJ	ET
LN	60	-	-	-
INY	-	60	-	-
GJ	-	-	60	-
ET	-	-	-	60
Fishmeal	9	9	8	8
Soyabean meal	6	6	6	6
Wheat bran	23.5	23.5	24.5	24.5
Oyster shell	1.00	1.00	1.00	1.00
Common salt	0.25	0.25	0.25	0.25
Vitamin-Trace mineral premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Nutrient composition (Calculated)				
CP, (%)	17.50	17.50	17.00	17.00
Ca, (%)	0.81	0.81	0.80	0.80
P, (%)	0.72	0.72	0.71	0.71
Lysine, (%)	0.94	0.94	0.95	0.95
Tryptophan, (%)	0.19	0.19	0.21	0.21
DE (kcal/kg)	3184	3184	3176	3176

Vitamin Trace Mineral Premix: Inclusion rate is 2.5g/kg to supply Vit. A = 8000 IU, Vit. D = 500 IU, Vit. E = 2.5 mg, Vit. K_3 = 1mg, Vit. B_2 = 2 mg, Vit. B_{12} = 0.005 mg, Folic Acid = 0.5 mg, Nicotinic Acid = 8 mg, Calcium Panthotenate = 2 mg, Choline Chloride = 50 mg, Manganese = 50 mg, Zinc = 4 mg, Copper = 4.5 mg, Cobalt = 0.1 mg, Iodine = 1 mg, Selenium = 0.1 mg.

Housing and feeding

The pigs were housed individually in concrete-floored wire mesh cages measuring 160 x 65 x 103 cm. The cages were located in roofed pens measuring 365 x 315 x 100 cm and each pen had four of the individual cages. Wooden feed and concrete water troughs were provided in each cage. Feed and water were provided *ad libitum.* Feeding was terminated and pigs were slaughtered when each pig attained a liveweight of 70 + 0.5 kg at the weekly weighing.

Parameters measured

During the experiment, weekly feed intake and weekly weight gains were recorded and corresponding average daily feed intake and average daily weight gain were calculated. The experimental pigs were removed and slaughtered for carcass evaluation after each pig attained a liveweight of 70 + 0.5 kg at the weekly weighing. The pigs were stunned, bled, scalded, singed and eviscerated. The dressed weights and weights of the viscera, head, trotters and the internal organs were recorded on the day of slaughter. The eviscerated carcasses were chilled in a coldroom at a temperature of 4^{o}C for 24 hours and other parameters taken.

Chemical and Statistical Analyses

The proximate compositions of the four maize varieties and diets were determined using procedures outlined by AOAC (1990). All data collected were subjected to analysis of variance using GenStat (Discovery Edition 3) and means separated by least significant difference.

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RESULTS AND DISCUSSION

Proximate composition of the maize varieties used

The proximate composition of the four maize varieties is shown in Table 2. The ET and INY maize varieties had almost the same levels of crude protein (8.10 vrs 7.90 %) while the GJ had a higher value (9.10 %) than the two mentioned earlier. However, the highest value of 10.0 % CP was obtained from the LN maize. Cromwell et al. (1983) reported similar higher values for normal maize but De Oliveira et al. (2011) reported 7.70, 9.87 and 7.36 % for common corn, high lysine corn and high oil corn respectively. These differences from De Oliveira et al. (2011) could be due to the differing environments in which the maize were cultivated and the variety as reported by Bressani et al. (1962). The GJ variety had higher ether extract content than the other three varieties and those varieties studied by O'Quinn et al. (2000) and De Oliveira et al. (2011). The dry matter content of the maize varieties were 85.0, 88.0, 85.0 and 86.0 % for the LN, INY, GJ and ET varieties respectively. These values are comparable to the values reported by Asche et al. (1985), O'Quinn et al. (2000) and De Oliveira et al. (2011). The ash percentages were lower than those recorded by De Oliveira et al. (2011).

Table 2 - Proximate composition (%) of the four maize varieties used in the experiment (as-fed basis)

Item	Maize variety			
	LN	INY	GJ	ET
Crude protein	10.0	7.9	9.1	8.1
Ether extract	5.5	3.0	7.0	5.5
Crude fibre	1.56	2.06	1.63	1.04
Ash	1.0	0.5	0.5	0.5
Moisture	15.0	12.0	15.0	14.0
Nitrogen free extract	66.94	74.54	66.77	70.86
Dry matter	85	88	85	86

Growth performance of the pigs

The summary of the growth performance of the pigs on the 4 dietary treatments is shown in Table 3. The mean total feed intake values were 205.6, 213.3, 207.90 and 207.2 kg for the LN, INY, GJ and ET diets respectively (Table 3).

Table 3 - Growth performance of pigs on the four dietary treatments

Parameter	Dietary treatment				LSD	Sign.
	LN	INY	GJ	ET		
No. of pigs	5	5	5	5	-	-
Mean initial weight, kg	13.3	13.2	13.3	13.2	1.368	NS
Mean final weight, kg	71.3	70.5	70.2	70.1	1.242	NS
Total feed intake, kg	205.6	213.3	207.9	207.2	14.28	NS
Mean daily feed intake, kg	2.27	2.26	2.06	2.19	0.272	NS
Mean weight gain, kg	56.9	56.9	57.3	58	1.724	NS
Average daily weight gain, kg	0.64^{a}	0.61^{a}	0.56^{b}	0.60^{ab}	0.079	*
Mean feed conversion ratio (feed/gain)	3.55	3.72	3.66	3.64	0.206	NS
Mean duration (days)	91.	95.2	102.2	95.2	14.17	NS
[g]Feed cost/kg, GH¢	0.49	0.51	0.48	0.48	-	-
Feed Cost/kg liveweight gain, GH¢	1.74^{b}	1.90^{a}	1.76^{b}	1.75^{b}	0.101	*

LSD-Least significant difference; Sign.-Level of significance; a,b: Values in the same row with different letters are significantly different (P<0.05).
[g]GH¢1 is equivalent to US$ 0.52

These values and the corresponding mean daily feed intakes of 2.27, 2.26, 2.06 and 2.19kg were not significantly (P > 0.05) different. The similarities in feed intake confirm that the energy content of the diets were similar as pigs eat to satisfy their energy requirements (Pond et al., 1995). The average daily weight gains (ADG) were 0.64, 0.61, 0.56 and 0.60 kg for LN, INY, GJ and ET diets respectively (Table 3). There were significant (P<0.05) differences among the treatment means with the LN, INY and ET values being similar but higher (P<0.05) than the value for the GJ diet. Rosa et al. (1977) stated that pigs fed Opaque-2 maize tended to grow slower than those fed non-opaque 2 maize but the differences in growth rate were not significant (P<0.05). Sullivan et al. (1989) had subsequently asserted that QPM diets reduced growth rate of starter pigs compared with pigs fed normal maize. Cromwell et al. (1969), Asche et al. (1985), Burgoon et al. (1992), Okai et al. (2001a, 2001b and 2007), De Oliveira et al. (2011), did not observe significant (P>0.05) differences in the ADG. However, Cromwell et al. (1983) and Osei et al. (1999) reported improved ADG of pigs fed QPM diets compared to normal maize diets. The differences in these findings may be attributable to the composition of diets and varieties of the maize used in these experiments. The feed conversion ratios were 3.55, 3.72, 3.66 and 3.64 for the LN, INY, GJ and ET diets respectively. It is apparent that the dietary treatments did not influence this parameter. Okai et al. (2001a, 2001b) had reported similar non-significant results when diets containing normal maize and Obatanpa (QPM) were fed to

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growing-finishing pigs. On the other hand, Maner et al. (1971) and Osei et al. (1999) reported results which showed improved FCE with the use of QPM varieties.

Feed Cost and Economy of Gain

The costs of the various diets were GH¢0.49, GH¢0.51, GH¢0.48 and GH¢0.48/kg for the LN, INY, GJ and ET diets respectively (Table 3). The reduction in the feed costs of the GJ and ET diets was due to the reduction in the fish meal inclusion levels in the diets in view of higher lysine and tryptophan levels in the GJ and ET. The reduction in fishmeal levels apparently had no (P>0.05) detrimental effects on the main performance parameters studied i.e. feed intake, feed conversion efficiency, growth rate and carcass dressing yield. In this study, the feed cost was reduced in the QPM-based diets i.e. GJ and ET up to GH¢10.00 per metric tonne. A similar observation had earlier been made by Osei et al. (1999). They stated a reduction of US$21.00 per metric tonne when QPM was incorporated in broiler diets owing to a reduction in the fishmeal levels in the diets. The feed cost per kg liveweight gain values were GH¢ 1.74, GH¢ 1.90, GH¢ 1.76 and GH¢ 1.75 for LN, INY, GJ and ET diets respectively (Table 3). There were significant (P < 0.05) differences among treatment means with the feed cost per kg liveweight gain being higher (P<0.05) for the INY group than the rest due to the higher price of the INY (i.e. GH¢0.55/ kg vrs GH¢0.50/kg for the GJ, ET maize). The values for this parameter for the LN, GJ and ET diets were similar (P > 0.05).

Carcass traits

The summary of the mean carcass traits for the pigs fed the four dietary treatments are shown in Table 4. There were no significant (P > 0.05) differences among treatment means of the various diets for the final weight, dressed weight and dressing percentage. These observations confirm earlier findings by Okai et al. (2001a, 2001b) and De Oliveira et al. (2011). As shown in Table 4, there were no significant (P > 0.05) differences among the treatment means for the shoulder, loin, belly and thigh weights. These results are similar to those of Okai et al. (2001a, 2001b and 2007) when Obatanpa (QPM variety) and normal maize varieties were used in grower-finisher diets of pigs. Earlier, Cromwell et al. (1969) had similar results and concluded that pigs on normal or high lysine corn diets formulated on an equal lysine-basis produced the similar growth performance in weanlings, and the similar growth rates and meat quality in growing-finishing pigs. The results again tallied with the works of De Oliveira et al. (2011). They found no differences in all carcass parameters measured in pigs fed diets containing common corn, high lysine corn and high oil corn.

The mean carcass length and backfat thickness values were not affected (P>0.05) by the dietary treatments (Table 4). Again, this finding agrees with the results of previous studies (Okai et al. 2001a, 2001b, 2007 and De Oliveira et al. 2011). With respect to standards, the values fell within grade 3 category of USDA (1985) stipulations for pork carcass and above the maximum backfat thickness of 2.80 cm, a standard for pork carcass fat thickness (Sterle, 2000). Nevertheless, the backfat thickness values apparently met the guidelines for the regulation of livestock products by FDL (1992).

Table 4 - Carcass traits of pigs fed the 4 diets

Parameter	Dietary treatment				LSD	Sign.
	LN	INY	GJ	ET		
No. of pigs	5	5	5	5	-	-
Mean live weight, kg	71.3	70.5	70.2	70.1	1.242	NS
Mean dressed weight, kg	52.93	52.87	53.22	52.49	2.039	NS
Mean dressing %	74.22	74.98	75.8	74.87	2.032	NS
Mean chilled dressed weight, kg	51.59	51.11	51.82	51.69	2.025	NS
Mean chilled dressing %	72.34	72.49	73.81	72.87	1.951	NS
Mean carcass length, cm	72.48	72.78	73.22	72.94	1.882	NS
Mean shoulder weight, kg	4.01	3.92	4.14	3.98	0.481	NS
Mean loin weight, kg	6.46	6.43	6.48	6.53	0.699	NS
Mean belly weight, kg	4.57	4.69	4.81	4.53	0.361	NS
Mean thigh weight, kg	6.45	6.47	6.2	6.4	0.4	NS
Mean backfat thickness, cm	3.18	3.25	3.07	3.14	0.449	NS

LSD= Least significant difference, Sign.= Level of significance (P < 0.05)

CONCLUSION

The results from the studies suggest that, the reduction in the inclusion levels of fish meal in the QPM diets (GJM and ETM) resulted in economic savings of GH¢ 10.00 per metric tonne. All carcass parameters were similar for all the dietary treatments but GJM and ETM diets gave slightly lower values in backfat thickness in the carcasses of the pigs. The studies also revealed that rats fed the GJM diet out-performed their counterparts in all the parameters measured. It can therefore, be concluded that the use of GJM and ETM varieties may offer an advantage of economic savings in the production of pork in Ghana.

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IN VITRO RUMINAL PROTEIN DEGRADABILITY OF LEAVES FROM THREE TREE SPECIES HARVESTED AT TWO CUTTING INTERVALS

A. EDWARDS[1]*, V. MLAMBO[1], C.H. OCTAVIUS LALLO[2], G. WAYNE GARCIA[2], M. DIPTEE[3]

[1]Department of Food Production, Faculty of Science and Agriculture, University of the West Indies, Hodge Street, St Augustine, Trinidad and Tobago
[2]The Open Tropical Forage-Animal Production Laboratory [Otp-Apl], Department of Food Production, Faculty of Science and Agriculture, University of the West Indies, St Augustine, Trinidad and Tobago
[3]School of Veterinary Medicine, Faculty of Medical Sciences, Mt Hope, Trinidad and Tobago

*Email: andell_e@hotmail.com

ABSTRACT: *In vitro ruminal protein degradation characteristics of protein supplements represent an accurate measure of the quality of protein for ruminant animals. As such, crude protein disappearance of Gliricidia sepium, Leucaena leucocephala and Trichanthera gigantea leaves, which are potential sources of supplemental protein for ruminants, was determined using the ANKOM in vitro ruminal degradability technique. Dry matter (DM) and crude protein (CP) disappearance were measured after 0, 2, 4, 6, 12, 24, 36, 48 and 72 h of incubation. Degradation kinetics were described using the Ørskov and McDonald equation* $y = a + b\left(1 - e^{-cx}\right)$*. The degradable part of the insoluble DM fraction (b) was highest (P<0.05) in G. sepium leaves (27%) at the 12 week cutting interval. Effective dry matter degradability (EDMD) was highest (P < 0.05) in the leaves of G. sepium (74.9%) at the 12-week cutting interval. CP washing losses was highest (P < 0.05) in the leaves of L. leucocephala (46.8%) and lowest in T. gigantea leaves (16.3%) at the 6-week cutting interval. Crude protein disappearance was highest (P<0.05) in the leaves of G. sepium and lowest in T. gigantea leaves at both the 6 and 12-week cutting intervals after incubation at 48 h. It is concluded that in vitro ruminal protein degradability is more pronounced in the leaves of G. sepium and L. leucocephala. Approximately 50% of their protein is degraded in the rumen suggesting that they would be useful as sources of readily available nitrogen for rumen microbes challenged with low nitrogen, fibrous basal diets. Trichanthera gigantea leaves have higher levels of rumen undegradable protein suggesting that they can be used to supply by-pass protein for animal.*

Key words: *In Vitro Rumen Degradability, Protein Quality, Effective Degradability, Harvesting Frequency, Tree Forages*

ORIGINAL ARTICLE

INTRODUCTION

Protein-rich forages are critical to ruminant livestock production particularly in developing countries where the quantity and quality of available basal diets fluctuates wildly in response to seasonal rainfall patterns. Tree forages can be used as protein supplements to these diets. As supplements, they supply ruminal microorganisms with a readily available source of nitrogen (N) that enables them to breakdown basal diets efficiently (Mcleod and Minson, 1969; Getachew et al., 1994). Livestock producers are interested in the quality and quantity of protein that these supplements supply. The extent of protein degradation in the rumen gives a measure of the available nitrogen to microorganisms and by-pass protein to the small intestine (Promkot and Wanapat, 2003). Protein quality for ruminants can be determined through the use of rumen degradability characteristics of the protein, especially the ratio of rapidly degradable (soluble) protein to rumen undegradable protein (Crawford et al., 1978). This is because for high producing ruminants, microbial protein alone may be inadequate to meet protein requirements without by-pass (rumen undegradable) protein supply. Proteins with a large rumen soluble N fraction will supply a ruminant animal with little by-pass protein (Crawford et al., 1978). On the other hand, a protein with large rumen undegradable protein fraction will be unable to supply sufficient N to rumen microbes resulting in reduced fermentation and hence poor utilization of the basal diet. Ruminal degradability techniques are therefore useful for characterizing forage protein in terms of its susceptibility to ruminal breakdown. Orskov and McDonald (1979), De Boer et al. (1986) and Chumpawadee et al. (2005), among other scholars, have presented the *in sacco* nylon bag technique as one of the most popular ways of evaluating the extent and pattern of degradability of feed protein in the rumen. Indeed, rate of disappearance, rapidly fermentable fraction, effective degradability and

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potential degradability of feed protein can be estimated successfully using the *in sacco* nylon-bag technique (Getachew et al., 1998). Cone et al. (2002) also indicated that the *in sacco* nylon-bag technique is the standard method for estimating the volume of protein escaping rumen fermentation in protein evaluation systems for ruminants. However, the *in sacco/in situ* approach lacks capacity to evaluate a large number of forage samples, requiring a large number of fistulated animals whose rumens can be used to incubate feed samples in nylon bags. This study used an *in vitro* rumen fermentation system based on the Daisy^{II} Incubator (ANKOM TECHNOLOGY, MACEDON NEW YORK) to estimate ruminal degradability of tree leaves. The Daisy^{II} Incubator employs ANKOM filter bag technology and simulates and simplifies the *in sacco* rumen degradability technique. A large number of forage samples (100 +) can be processed in one batch. *Gliricidia sepium, L. leucocephala* and *T. gigantea* are three protein-rich forages grown in Trinidad and Tobago. Researchers have since recognize their importance as livestock feeds and are currently evaluating them at various levels. *Leucaena leucocephala* originated from Central America and Mexico and it belongs to the Mimosaceae family (Batson et al., 1987; Shelton and Brewbaker, 1994; Garcia et al., 1996). The shrub thrives well in alluvial and heavy clay soils. However, it has been found growing in saline soils (Batson et al., 1987). *Gliricidia sepium* is native to Mesoamerica and it's a member of the Fabaceae family (Simons and Stewart, 1994). Though recognized as essential forage in many parts, its use has been limited by palatability and toxicity concerns (Simons and Stewart, 1994). *Trichanthera gigantea* is native to Columbia and it belongs to the Acanthaceae family (McDade, 1983). The tree is adapted to the humid tropics and it is capable of thriving in acid (pH 4.5) and poor soils where there is good drainage.

There is a paucity of information as it relates to the ruminal degradability characteristics of these forages at different harvesting stages. Hoffman et al. (1993) reported that maturity stage of forage trees can influence DM and CP degradation fractions and degradation rates. Such information can be used to make informed decisions on how to incorporate the tree leaves into the diets of animals. This study, therefore, seeks to determine the *in vitro* ruminal dry matter and protein degradation parameters for *G. sepium, L. leucocephala* and *T. gigantea* leaves harvested at 6 and 12-week cutting intervals.

MATERIALS AND METHODS

Study site

Leaf samples were obtained from established tree species at the University of the West Indies Field Station (UFS). The UFS (Lat 10° 38' N Lon 61° 23' W) has a relatively flat topography with an altitude of 15.2 meters above mean sea level. Average annual rainfall is 1782.9mm with an average monthly temperature of 27 °C. The soil type is river estate loam. The soil is free draining with a pH range of 5.0 – 6.2.

Sample preparations

Fresh leaf materials (leaves with petioles) were harvested from forage tree species (*L. leucocephala, G. sepium,* and *T. gigantea*) that were trimmed to a height of 1 meter at UFS. Harvesting was done in the morning manually by cutting branches at a distance of 1 m from the growing tip for (*G. sepium*) and 0.5 m for (*T. gigantea* and *L. leucocephala*), 6 and 8 weeks after the trees had been trimmed to a 1 meter height. Leaves from six individual trees for each species were harvested, weighed and stored into brown paper bags separately. Leaf samples were immediately transported to the laboratory and oven dried to a constant weight at 65 °C. The dried samples were then milled to pass through a 1mm sieve using a Wiley Mill (GLEN CRESTON LTD, MIDDLESEX, UK) and kept in separate brown paper bags pending chemical analysis and *in vitro* ruminal fermentation.

Chemical analyses

Chemical analyses were carried out as part of an earlier study (Edwards et al., 2012). Dry matter, organic matter, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin, soluble and insoluble condensed tannin content of leaves were determined. The chemical composition of the leaves is presented in Table 1 below to further describe the substrates fermented in this study.

In vitro ruminal dry matter and crude protein degradation

The Daisy^{II} Incubator (ANKOM TECHNOLOGY, MACEDON NEW YORK) was used to measure kinetics of DM and CP degradation of *G. sepium, L. leucocephala* and *T. gigantea* leaves. Milled leaf substrates (0.5 g) were weighed into filter bags (F 57) that had been pre-rinsed in acetone. Heat sealed bags were placed in the Daisy^{II} Incubator digestion jars. Sealed blank bags were included to enable the calculation of the blank bag correction factor. About 1 600 ml of ANKOM buffer (ANKOM TECHNOLOGY, MACEDON NEW YORK) was added to each digestion jar. Digestion jars with bags and buffer solution were placed into the Daisy^{II} Incubator set at 39°C and allowed to equilibrate for 30 minutes. Ruminal fluid was collected at 8:00 am. The donor was a crossbred Holstein heifer that was offered tanner grass, *G. sepium, L. leucocephala, T. gigantea* leaves and dairy concentrate (MASTER MIX FEEDS LTD, TRINIDAD). Rumen digesta from multiple sites within the rumen was sampled by hand and the rumen fluid squeezed into a prewarmed thermos flask. It was then transported to the laboratory, blended and strained through two layers of warm cheese cloth. The strained rumen fluid was held under carbon dioxide at 39 °C. Digestion jars were removed from the incubator, one at a time, and 400 ml of rumen fluid inoculum was added to each jar. Inoculated digestion jars were purged with CO_2 for 30 seconds after which they were sealed and returned into the

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incubator. All bagged samples were placed in the jars at the start of the incubation period and were than sequentially withdrawn at 2, 4, 6, 12, 24, 36, 48 and 72 h. After each withdrawal, bags were thoroughly rinsed with cold tap water until the water was clear. Time 0 h samples were not incubated but were washed in cold water to determine solubility at time 0 h. After rinsing, bags were placed in the ANKOM^{200} Fiber Analyzer and the procedure for NDF determination was followed, that is, samples were refluxed with neutral detergent solution for 1hr according to Van Soest et al. (1991).

Calculations

In vitro ruminal DM degradability was determined using the following formula:

$$\%\ IVTD\ (DM\ basis) = 100 - (W3 - (W1\ x\ C1))\ x\ 100/(W2\ x\ DM)$$

Where: W1 = Bag tare weight, W2 = Sample weight, W3 = Final bag weight after *In vitro* and sequential ND treatment, C1 = Blank bag correction factor (final oven-dried weight÷ original blank bag weight).

In vitro ruminal CP disappearance was calculated by subtracting the CP content of the degraded residue at each incubation time from the CP content of samples before degradation.

The DM and CP degradation data were fitted, using Datafit 9 (OAKDALE ENGINEERING) to the exponential equation (Ørskov and Mc Donald, 1979): $Y = a + b\left(1 - e^{-cx}\right)$

Where, *y* is the disappearance of DM or CP during time t; *a* is the rapidly soluble fraction (washing losses); *b* is the degradable part of the insoluble fraction; *c* is the rate of degradation of fraction b; and *t* is time of incubation. Potential degradability was calculated as *a+b*. The effective degradability of DM (EDDM) was calculated using the equation below, after assuming a ruminal fractional outflow rate (r) of 2 %/h at maintenance feeding levels.

$$EDDM = a + (bc)/(c + r)$$

where: r is the estimated rate of outflow from the rumen and a, b, and c are the parameters described in the Ørskov and McDonald exponential equation above.

Statistical Analysis

Data of DM and CP disappearance, degradation kinetics were analyzed using the general linear model (GLM) procedure of MINITAB (version 15) according to the following model:

Y = μ + D + F + D*F + e

where: Y = dependent variable, μ = overall mean, F = species effect (*G. sepium, L. leucocephala* and *T. gigantea*), D = cutting interval effect (6, 12-week), F*D = species*cutting interval effect and e = residual error.

Table 1 - The effect of species and cutting interval (weeks) on the chemical composition (g/kg DM) of *Gliricidia sepium, Leucaena leucocephala* and *Trichanthera gigantea* at UFS (Edwards et al., 2012)

Item / Species	Cutting interval	Chemical components[1]								
		DM	OM	CP	ADIN	ADF	NDF	ADL	SCT	ICT
G. sepium	6	895^{a}	915^{a}	284^{a}	34^{a}	405^{a}	582^{a}	22^{a}	0^{a}	0
	12	911^{a}	907^{a}	257^{a}	27^{a}	438^{a}	577^{a}	26^{a}	0^{a}	0
L. leucocephala	6	907^{b}	918^{a}	318^{b}	37^{b}	539^{b}	609^{b}	33^{b}	0.2^{b}	0
	12	921^{b}	913^{a}	268^{a}	34^{b}	491^{b}	597^{b}	32^{b}	0.2^{b}	0
T. gigantea	6	859^{c}	739^{b}	226^{c}	38^{b}	549^{b}	622^{b}	25^{c}	0^{ac}	0
	12	877^{c}	737^{b}	185^{b}	30^{c}	541^{c}	648^{c}	26^{ac}	0^{ac}	0
Species		***	***	***	***	***	***	***	***	NS
Cutting interval		**	NS	***	**	NS	NS	NS	NS	NS
Species*Cutting interval		NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a,b,c} Means within a column with different superscripts differ significantly (P<0.05). *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant. [1]Chemical components: DM = dry matter, OM = Organic matter, CP = Crude protein, ADIN = Acid detergent insoluble nitrogen, ADF = Acid detergent fibre, NDF = Neutral detergent fibre, ADL = Acid detergent lignin, SCT = Soluble condensed tannins, ICT = Insoluble condensed tannins.

RESULTS

In vitro ruminal DM degradability

Dry matter disappearance (DMD) data are presented in Table 2. Dry matter washing losses were highest (P<0.05) in *L. leucocephala* leaves (74%) and lowest in *T. gigantea* leaves (59%) at the 6 week cutting interval. A similar ranking of species with regards to DM washing losses was also observed at the 12-week cutting interval. Dry matter disappearance after 36 h of incubation was lowest (P<0.05) in *T. gigantea* leaves at the 6-(67.8) and 12-week-(67%) at cutting interval. At 48 h incubation, DMD was highest (P<0.05) in the leaves of *G. sepium* (83%) and lowest in *T. gigantea* leaves (68%) at the 6 week harvesting interval (Table 2). Similarly, DMD was highest (P<0.05) in the leaves of *G. sepium* (77.5%) and lowest in *T. gigantea* leaves (67%) at the 12 week harvesting interval. The rapidly soluble DM fraction (*a* fraction), the degradable part of the insoluble DM fraction (*b* fraction), rate of DM degradation of fraction *b* (*c*) and potential DM degradation (*a+b*) are presented in Table 3. The rapidly soluble DM fraction (a) was highest (P<0.05) in *L. leucocephala* leaves (73%) and lowest in the leaves of *T. gigantea* (59 %) at the 6 week harvesting interval (Table 3). Similarly, the rapidly soluble DM fraction (a) was highest (P<0.05) in *L. leucocephala* leaves (64.9%) and lowest in the leaves of *T. gigantea* (56.4%) at the 12-week harvesting interval

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(Table 3). The degradable part of the insoluble DM fraction (*b*) was highest (P<0.05) in *L. leucocephala* leaves (17.7%) at the 6 week cutting interval. The *b* fraction was highest (P<0.05) in *G. sepium* leaves (27%) at the 12 week cutting interval. The rate of DM degradation of fraction *b* (*c*) was lowest (P<0.05) in the leaves of *G. sepium* (2%/h) at the 12 week cutting interval. Potential DM degradation (*a+b*) was highest (P<0.05) in *L. leucocephala* leaves at 6-week (90.7%) and 12-week (77.4%) cutting intervals (Table 3). Effective dry matter degradability (EDMD) was highest (P<0.05) in the leaves of *G. sepium* (74.9%) at the 12-week cutting interval (Table 3).

In vitro ruminal protein degradability

In vitro ruminal CP disappearance data are presented in Table 4. CP washing losses was highest (P<0.05) in the leaves of *L. leucocephala* (46.8%) and lowest in *T. gigantea* leaves (16.3%) at the 6-week cutting interval. A similar trend followed where CP washing losses was highest (P<0.05) in the leaves of *L. leucocephala* (43.3%) and lowest in *T. gigantea* leaves (12.4%) at the 12-week cutting interval. Crude protein (CP) degradability at 24 h incubation was lowest (P<0.05) in *T. gigantea* leaves at the 6-(13.7%) and 12-week-(30.9 %) harvesting interval (Table 4).

At 36 h incubation time, CP disappearance was highest (P<0.05) in *G. sepium* leaves at 6-week (48%) and 12-week (49%) harvesting intervals. At the 48 h incubation CP disappearance was highest (P<0.05) in the leaves of *G. sepium* (60%) and lowest in *T. gigantea* leaves (29%) at the 6-week cutting interval. A similar trend followed where CP disappearance was highest (P<0.05) in the leaves of *G. sepium* (50%) and lowest in *T. gigantea* leaves (27%) at the 12-week cutting interval. The convergence criterion for the Ørskov and McDonald nonlinear model was not met for the degradable part of the insoluble CP fraction (*b*) and the rate of CP degradation of fraction *b* (*c*) for all species. As a result mean CP degradability values per incubation time are presented in Table 4.

DISCUSSION

In vitro ruminal DM degradability

Dry matter disappearance increased with increasing incubation time (Table 2). This is consistent with reports by Kirkpatrick and Kennelly (1987) which showed increases in DM and CP disappearance of barley (*Hordeum vulgare*), canola meal and soybean meal and Paya et al. (2008) who recorded increases in DM and CP disappearance of corn grain, soybean meal, wheat bran and alfalfa (*Medicago sativa*) with increasing incubation time. Though not statistically significant, DMD was lower at the 12 week cutting interval for all species. Dry matter disappearance was highest in *G. sepium* leaves and lowest in the leaves of *T. gigantea* at both harvesting intervals suggesting that the DM in *G. sepium* leaves is highly degradable. In addition, this can be due to lower fibre fractions (NDF, ADF) in the leaves of *G. sepium* (Table 1) as *in situ* DM disappearance is positively correlated with reducing sugars and negatively correlated with NDF (Vitti et al., 2003). The lower DM degradability in *T. gigantea* leaves can be attributed to its higher fibre content (NDF, ADF) (Table 1) as acid detergent fibre is negatively correlated with DM degradability (Smith et al. 1991).

Table 2 - *In vitro* ruminal dry matter disappearance (%) of *G. sepium, L. leucocephala* and *T. gigantea* at 6 and 12 weeks cutting intervals

Item / Species	Cutting Interval	Incubation period (h)								
		0	2	4	6	12	24	36	48	72
G. sepium	6	65^{aA}	65^{a}	66^{a}	68^{a}	68^{a}	76^{a}	80^{a}	83^{a}	85^{a}
	12	61^{bA}	56^{b}	65^{a}	66^{a}	67^{a}	73^{a}	76^{b}	77.5^{b}	82^{b}
L. leucocephala	6	74^{aB}	72^{c}	73.5^{b}	75^{c}	75^{b}	77^{a}	79^{ac}	81^{c}	82^{b}
	12	63^{bB}	66.5^{a}	67.4^{c}	69^{a}	69^{ac}	69^{ab}	73^{d}	74^{d}	74.7^{c}
T. gigantea	6	59^{aC}	61^{d}	59.5^{d}	61^{d}	62^{d}	62^{c}	67.8^{e}	68^{e}	75^{c}
	12	58^{aC}	59^{d}	58^{d}	58^{e}	60^{e}	69^{ab}	67^{e}	67^{e}	68.5^{d}
Species		***	NS	***	***	***	NS	***	***	***
Cutting interval		***	NS	*	*	*	NS	*	*	***
Species*Cutting interval		**	NS	NS	NS	NS	NS	NS	NS	NS
SEM		1.3	3.9	1.5	1.6	1.7	4.2	1.9	1.9	1.5

In a column where Species*CI is significant, lowercase superscripts compare CI means within species, while uppercase superscripts compare species for each CI. In columns where Species*CI is not significant, CI means are compared across species. Means within a column with different superscripts (a - e) differ (P < 0.05), *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant.

The rapidly soluble DM fraction (*a*) values at the 6 and 12-week cutting intervals for all species were higher than those reported by Kirkpatrick and Kennelly (1987), Promkot and Wanapat (2003), Chumpawadee et al. (2005), Paya et al. (2008) and Ilghami et al. (2008) who used different forage species. The soluble DM fraction (*a)* was highest in *L. leucocephala* leaves which indicates faster initial rate of degradation when compared to the other species. This is attributed to the fact that high soluble fractions make feeds more degradable as microorganisms are able to attach more readily to the soluble fractions (Chumpawadee et al., 2005). The *c* values for all species were similar to those reported by Promkot and Wanapat (2003), Chumpawadee et al. (2005) and Ilghami et al. (2008) but lower than those reported by Paya et al. (2008). Effective dry matter degradability (EDMD) values at 6 and 12-week cutting intervals for all species were higher than those reported by Promkot and Wanapat (2003), Chumpawadee et al. (2005), Paya et al. (2008) and Ilghami et al. (2008). Effective dry matter degradability (EDMD)

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was lowest in the leaves of *T. gigantea* possibly due to its high fibre content (NDF, ADF, ADL) (Table 1). High fibre suggests that less nitrogen would be available for rumen microbes hence reduce degradability due to lower microbial activity. Kamalak et al. (2005) reported that *in situ* DM degradability and estimated parameters were negatively correlated with NDF and ADF but positively correlated with CP content of tumbleweeds (*Gundelia tournefortii*).

***In vitro* ruminal protein degradability**

Crude protein disappearance increased with increasing incubation time in the leaves of *G. sepium* (Table 4). This is supported by Kirkpatrick and Kennelly (1987) who showed increases in DM and CP disappearance of barley (*Hordeum vulgare*), canola meal and soybean meal and Paya et al. (2008) who recorded increases in DM and CP disappearance of corn grain, soybean meal, wheat bran and alfalfa (*Medicago sativa*) with increasing incubation time. Cutting intervals had a minimal influence on the CP disappearance of the species. In a study where tumbleweed (*Gundelia tournefortii*) hays were harvested at three maturity stages, *in situ* DM Disappearance decreased with increasing maturity (Kamalak et al., 2005). Hoffman et al. (1993) reported that maturity stage of alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), rye grass (*Lolium perrene*) and timothy (*Phleum pratense*) affected DM and CP degradation fractions and degradation rates. Crude protein disappearance was highest in the leaves of *G. sepium* and lowest in *T. gigantea* leaves possibly influenced by the nature of the protein. Such data suggest that *T. gigantea* can be used to increase bypass protein or replace readily degradable protein sources in the diet owing to its low degradability by ruminal microbes (Ilghami et al., 2008). The lower degradability of CP in *T. gigantea* can also be attributed to its higher ADIN values (Table 1) in comparison to *G. sepium* suggesting that the majority of its protein may be bound to fibre thus rendering it insoluble and inaccessible by rumen microbes (Kirkpatrick and Kennelly, 1987).

The estimation of degradable part of the insoluble CP fraction (*b*) failed because the convergence criterion for the non-linear model was not met after several iterations using the Datafit (version 9) curve fitting programme (Table 5). This indicates that CP degradation profile did not closely fit the non-linear equation as a result mean degradation values are presented in Table 4. The rate of CP degradation of *b* (*c*) of all species was slower than those reported by Wang et al. (2009) Ximena Valderrama and Rene Anrique (2011). The rate of CP degradation was slowest in *G. sepium* leaves at cutting intervals 6 and 12-week. This may be due to the CP in *G. sepium* leaves having associations with other structural components (fibre) hence lowering the availability to microbial attack (Kohn and Allen, 1995).

Table 3 - *In vitro* ruminal dry matter degradation parameters of *G. sepium, L. leucocephala* and *T. gigantea* at cutting intervals 6 and 12 week

Species \ Item	CI[1]	Degradation parameters				
		a[2] (%)	b[3] (%)	c[4] (%/h)	a+b[5] (%)	EDDM[6] (%)
G. sepium	6	65[a]	NC[7]	0.02[a]	NC	NC
	12	61[b]	27[a]	0.02[a]	88.3[a]	74.9[b]
L. leucocephala	6	73[c]	17.7[a]	0.01[a]	90.7[a]	79.4[ac]
	12	64.9[d]	12.5[a]	0.05[b]	77.4[a]	72[d]
T. gigantea	6	59[e]	NC	0.00[a]	NC	NC
	12	56.4[f]	NC	0.04[ab]	NC	NC
Species		***	NS	NS	NS	***
CI		**	NS	NS	NS	***
Species*CI		NS	NS	NS	NS	NS
SEM		1.8	2.15	0.02	2.15	1.3

[a-f] Means with different superscripts in a column differ significantly (P<0.05). *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant. [2]a = the rapidly soluble fraction; [3]b = the potentially degradable fraction; [4]c = the rate of degradation of fraction b; [5]a+b = potential degradation; [6]EDDM = effective degradability of DM, [1]CI = cutting interval, NC[7] = non convergence

Table 4 - *In vitro* ruminal crude protein disappearance (%) of *G. sepium, L. leucocephala* and *T. gigantea* at 6 and 12 weeks cutting intervals.

Species \ Item	Cutting Interval	Incubation period (h)								
		0	2	4	6	12	24	36	48	72
G. sepium	6	41.8[a]	49.8[a]	38.7[a]	45.4[a]	45[a]	46.2[a]	48[a]	60[a]	59[a]
	12	31.3[b]	40.3[b]	34.8[a]	39.7[a]	42.8[a]	43[a]	49[a]	50[b]	74[b]
L. leucocephala	6	46.8[c]	51.2[a]	52.5[b]	53[b]	47.5[a]	42.7[a]	37[b]	44[c]	42[c]
	12	43.3[c]	44.6[ab]	42.7[c]	41.9[c]	39.8[ab]	44.8[a]	41[b]	43[c]	37[d]
T. gigantea	6	16.3[d]	7.5[c]	9.8[d]	9.8[d]	25.2[c]	13.7[b]	23[c]	29[d]	29[e]
	12	12.4[d]	11.5[c]	8.3[d]	16[e]	22.4[c]	30.9[c]	29[d]	27[d]	32[f]
Species		***	***	***	***	*	**	***	***	***
Cutting interval		NS	NS	NS	NS	NS	NS	NS	*	NS
Species*Cutting interval		NS	NS	NS	NS	NS	NS	NS	NS	NS
SEM		5.7	6	6.2	5.5	6.2	6	5.1	2.2	

[a-f] Means with different superscripts in a column differ significantly (P < 0.05). *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant.

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CONCLUSION

The results of this study demonstrated that approximately 50% of CP in the leaves of *G. sepium* and *L. leucocephala* could be degraded in the rumen. This indicates that these protein trees can supply a readily available source of N to rumen microbes that have to ferment poor quality grass basal diets. Crude protein disappearance was least in *T. gigantea* leaves which suggest that it can be used supply by-pass protein to the duodenum of the ruminant animal. a feeding strategy where *T. gigantea, G. sepium and L. leucocephala* leaves are combined and offered as protein sources could ensure that both rumen microbial N and by-pass protein requirements are met.

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PRELIMINARY ON–STATION STUDY OF GROWTH PERFORMANCE OF GROWER PIGS ON ENSILED CASSAVA PULP AND DRIED CASSAVA LEAVES

S.W.A. RHULE, P. ASIEDU, G.Y. AMELEKE, R.Y. BAIDEN, E.T. SOTTIE, H.R. OTSYINA

Csir-Animal Research Institute, Box Ah 20, Achimota-Accra, Ghana

Email: pierroboakye@yahoo.com

ABSTRACT: *The performance of grower pigs on diets containing graded levels of cassava pulp, cassava peels and dried cassava leaves was studied. Twenty-four Large White grower pigs at an average initial live-weight of 20 kg were distributed over six diets by the completely randomized design. The pulp was preserved by ensiling in polyethylene bags for a period of three months before use. The pigs were group-fed once-daily for five weeks. The average daily gains (ADG) of the pigs were 0.27, 0.19, 0.28, 0.26, 0.15 and 0.20 kg live-weight gain/day on diets 1, 2, 3, 4, 5 and 6 respectively. The cost of feed were 0.16, 0.15, 0.15, 0.13, 0.12 and GH¢0.10 per kg of feed for diets 1, 2, 3, 4, 5, and 6 respectively. The corresponding economy of gain (EG) were 0.58, 0.74, 0.53, 0.49, 0.72 and GH¢0.49. The highest inclusion rate was 30% for the pulp and 20% for the leaves. The pigs were weighed weekly over a five week period. Whereas the ADG of the pigs in this study was best on diet 3 (25% pulp), the EG was best on the diets 4 (30% pulp) and 6 (20% cassava leaves).*

ORIGINAL ARTICLE

Key words: *Ensiled Cassava Pulp, Dried Cassava Leaves, Large White Grower Pig, Average Daily Gain, Economic of Gain*

INTRODUCTION

The Ayensu Starch Company Factory (ASCo) at Bawjiase in the Central Region has a capacity to produce 20,000 MT of starch per year. With a cassava to starch ratio of about 4:1 it becomes apparent the quantities of pulp and by-products potentially to be generated by ASCo. Even with current production at below the installed capacity pollution of the environment around the factory is becoming a matter of great concern.

Initial attempts by some pigs farmers in the Central Region near ASCo at feeding the pulp to their animals resulted in casualties and the production of unacceptably fatty carcasses. However cassava and its by-products have been found to be potential replacement for maize as energy sources in pig diets (Fleischer, 1975; Sonaiya et al., 1982; Barnes and Oddoye, 1985; Sonaiya and Omole, 1983; Ogbonna and Oredein, 1998; Phuc and Lindberg, 2000; Phuc et al., 2000 and Rhule et al., 1998). Samples of pulp analysed in the laboratory of Animal Research Institute had average composition of 84.8% moisture, 4.80% ash, 0.38% ether extract, 2.56% crude protein and 3.51% crude fibre.

The high moisture content of the pulp at about 85% predisposes the pulp to very rapid deterioration resulting in reduced shelf-life. The initial step in the study was to evolve methods of preserving the material for it to be evaluated for pigs feeding.

The pulp has a low CP content just as cassava peels and whole cassava. Their substantial inclusion in pig diets would require good but low-priced sources of CP to provide for the requirements of the pigs. There are several oilseed cakes available in Ghana to be used to augment the protein level in the cassava-based diets (Rhule, 1996; Rhule, 1999). Dried cassava leaf has been found to be a good source of CP, minerals and vitamins. The protein of cassava leaf has been found to vary between 17.0 and 40.0% with 0.85 of the CP being true protein (Ravindran, 1993). Cassava leaf has higher content of most essential amino acids, apart from sulphur amino acids, than soyabean meal (Eggum, 1970; Gomez and Valdineso, 1984). Cassava leaves has been found to be a potential replacement for soya bean meal and fish meal in pig diets (Preston 2001). On the other hand there is a dearth of information on the use of pulp for feeding pigs.

The objectives of this study were to evolve a method of preserving the pulp for feeding over a period and determine safe levels of inclusion in pigs diets.

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MATERIAL AND METHODS

Preservation method

Freshly produced pulp was collected from the factory and ensiled at the Frafraha station of the CSIR-ARI. A plastic sheet was used for the process. The material was kept for a period of three months before being fed to the pigs. Sub-sample of the pulp was kept for a period of three months in tightly capped plastic sample bottles. Over the period, a bottle of sample was taken weekly and physically examined for growth of mould and colour changes after its pH had been recorded.

Animals

Twenty-four Large White grower pigs at an average initial live weight of 20 kg were distributed over the six diets. Each treatment was replicated four times with a pig per pen in a completely randomized design.

Treatments

Six diets were formulated incorporating the ensiled pulp and other cassava by-products. The six diets were made to be as similar as possible in the crude protein content. The composition of the diets is shown in Table 1. With the exception of the pulp, the respective ingredients of the various diets were mixed in bulk. The calculated dry equivalents of the pulp were weighed in the morning and mixed with the previously compounded diets before feeding.

The pigs were restricted-fed daily a ration equivalent to 5% of the total group-weight. Water was provided *ad libitum.* The pigs were individually weighed weekly. The weekly group feed allowances were adjusted after the weekly weighing and calculated on the total group live weight. The pigs were fed the respective diets over a five-week period.

Table 1 - Composition of Cassava-Pulp Diets fed to Grower Pigs (%)

Ingredient	Dietary treatments					
	1	2	3	4	5	6
Maize	33.30	-	-	-	-	-
Wheat bran	30.0	-	-	-	-	-
Cassava pulp	-	15.00	25.00	30.00	20.00	20.00
Whole cassava	-	20.30	12.30	7.30	9.30	5.30
Cassava peels	-	17.00	15.00	10.00	20.60	14.60
Cassava leaves	-	5.00	5.00	5.00	10.00	20.00
Palm kernel cake	30.00	30.00	30.00	30.00	30.00	30.00
Fishmeal	1.00	3.00	3.00	3.00	3.00	3.00
Soya bean meal	4.00	8.00	8.00	8.00	5.40	5.40
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Premix*	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00
Determined composition						
Moisture	10.97	44.32	61.30	63.62	50.75	48.64
Dry matter	89.05	55.68	38.70	36.38	49.25	51.36
Crude Protein	16.89	14.19	13.45	15.37	13.82	14.27
Ether Extract	10.34	5.46	4.70	9.21	6.94	6.06
Ash	4.80	8.89	5.81	8.59	8.59	7.73
Crude Fibre	9.20	18.03	18.64	23.37	20.69	21.96

*Composition of vitamin/mineral premix per kg: Vitamin E, 25mg; Vitamin A, 6250 IU; Vitamin D3, 1250 IU; Vitamin K3, 25mg; Vitamin B1, 25mg; Vitamin B2, 60mg; Vitamin B6, 40mg; Vitamin B12, 2mg; Elemental calcium, 25mg; Elemental phosphorus, 9mg; Elemental magnesium, 300mg; Iron, 400mg; Selenium 1.0mg, Iodine 20mg, Copper 60mg, Magnesium 100mg, cobalt 10mg, Zink, 150mg; Sodium Chloride, 1.5mg; Choline Chloride, 500mg; Live Lactobaccillus spore, 0.2 million cfu; Niacin, 40mg; Folic Acid, 10mg; d-Biotin, 5mcg.

Statistical analysis

Data was analysed statistically using analysis of variance (ANOVA) technique and SPSS version 16.0 (Steel et al., 1997).

RESULTS AND DISCUSSION

The analyzed composition of the diets is shown in Table 1. The diets containing the pulp had very high moisture levels compared to the control diet, being highest in diet 4. All the test D2 to D6 had similar CP levels and lower than the control (D1). The determined CP levels of the pulp diets could be considered low compared to D1 (NRC, 1998) with the ether extract (EE) of the control (D1) also being higher. Increasing levels of the pulp in the diets resulted in increasing level of the crude fibre in the diets and much higher than the value of 6.0% for pigs. The lower EE values of the diets with pulp would lead to lower energy values compared to the control. The performance of grower pig is shown in Table 2.

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Table 1 - The performance of grower pig fed cassava pulp based diets

Parameters	Dietary Treatment						
	1	2	3	4	5	6	SEM
Initial wt (kg)	20.00	20.50	20.50	21.25	21.00	20.50	0.76
Final wt (kg)	27.50	25.88	28.38	28.88	25.25	26.00	0.99
ADG kg/day	0.27a	0.19ab	0.28a	0.27a	0.15b	0.20ab	0.01
FCR kg feed/kg.l.wt gain	3.72	4.94	3.56	3.75	6.43	4.87	-
Unit cost/kg feed Gh₵	0.16	0.15	0.15	0.13	0.12	0.10	-
Economy of gain (Gh₵ kg l.wt gain)	0.58	0.74	0.53	0.49	0.72	0.49	-

Means in a column with similar or no superscript are not significantly different at P>0.05); SEM: standard error of means

Although there were no significant (P>0.05) differences in the final live-weights of the pigs on the diets, the ADGs of the pigs were found to be significantly (P<0.05) different being 0.27, 0.19, 0.28, 0.27, 0.15 and 0.20 kg/day on D1, D2, D3, D4, D5 and D6 respectively. The ADG of the pigs on D1, D3 and D4 were similar and significantly (P<0.05) higher than those pigs on D2 and D6, which were also similar. The pigs on D5 had the lowest ADG. Both D1 and D4 had the recommended CP values (NRC, 1998), hence the ADG observed. Diets 3 and 4 had the high inclusion levels of the pulp leading to higher CF values of 18.4% in D3 and 23.37% in D4 compared to the value of 9.2% in D1. The results indicated that the composition of CF in the pulp could be more important than only the level. Such observations had been made in previous studies (Sarwat et al., 1988; Eustace and Dorothy, 2001). There could also be a protein sparring influence from the energy of the pulp with the resultant ADG as observed, indicating optimum use of the protein for growth. The ADGs of the pigs indicated that 30% pulp and 5% dried cassava leaf would be the optimum for the grower pig. The ADGs on D3 and D4 being higher than D5 and D6 despite the higher CF could be attributed to the higher levels of the dried cassava leaf in the later diets. Cassava leaf contains saponins and tannins which are known to adversely affect the digestibility and absorption and utilization of the feed (Gohl, 1982; Bressani, 1993). The ADGs of 0.19 kg/d on D2 (14.19% CP) and 0.20 kg/d on D6 (14.27% CP) were similar. The combination of high levels of cassava peels and cassava leaf in D5 coupled with the low CP and high CF could have contributed to the observed ADG of the pigs on the diet. The ADG of the pigs on D1, D3 and D4 were, however ,considered lower than values obtained on similar studies (Tewe and Oke, 1983; Rhule, 1996; Rhule, 1998).

The FCR of the diets by the pigs are shown in Table 2. The values on D1, D3, and D4 were similar and higher than D2 and D6, which in turn were similar. Diet 5 had the lowest FCR. The highest FCR was obtained on D3. The FCR obtained on D3 was higher than values obtained on other studies (Rhule, 1996; Rhule, 2001).

There was a progressive decrease in the unit cost of the feed with increasing levels of both the cassava pulp and dried cassava leafs in the diets (Table 2), with as much as 20% reduction in the unit price. These were occasioned by the drastic reduction in the levels of both fishmeal and soyabean and the complete elimination of the expensive energy source, maize in the diet.

The economies of gain (EG) of the pigs on the diets are shown on Table 2. Diets 4 and 6 had the best and similar EG. Diet 4 had the highest inclusion level of 30% pulp while D6 had the highest inclusion levels of 20% cassava leaves.

CONCLUSION

The study indicated that ensiled cassava pulp, dried cassava leafs could completely replace maize in the diets of grower pigs. Whereas pigs on D3 had best ADG and FCR, EG was best on D4 and D6.

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NUTRITIVE VALUE OF RICE POLISH

M.E. HOSSAIN[1*], S. SULTANA[2], S.M.S. SHAHRIAR[3] and M.M. KHATUN[4]

[1]Department of Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh
[2]UG student, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh
[3]Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh
[4]Veterinary Officer, Strengthening of Support Services for Combating Avian Influenza Project-Bangladesh, District Livestock Office, Dhaka

*E-mail: emrancvasu@yahoo.com

ABSTRACT: *The present study was undertaken to observe the chemical composition of different types of rice polish available in different areas of Chittagong, Bangladesh. Twenty different types of rice polishes were collected from study areas. Chemical analyses of the samples were carried out in triplicate for moisture, dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extract (NFE), ether extract (EE) and total ash (TA) in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. Metabolizable energy (ME) was calculated mathematically for all samples by using standard formula. Results indicated that, there were no marked variations (P>0.05) in the moisture, DM and TA contents of the samples. However, ME, CP, CF, NFE and EE content significantly differed (P<0.01) from one sample to another. Moisture content varied from 4.0 to 11.4 g/100g, DM content varied from 88.6 to 96.0 g/100g, ME content varied from 1321.8 to 3086.9, CP content varied from 4.7 to 14.9 g/100g, CF content varied from 6.4 to 41.5 g/100g, EE content varied from 1.0 to 18.0 g/100g, NFE content varied from 25.1 to 52.9 g/100g and TA content varied from 7.1 to 17.6. It could therefore, be inferred that, the chemical composition rice polish currently available in the local market are widely variable.*

ORIGINAL ARTICLE

Key words: *Rice Polish, Moisture, Dry Matter, Crude Protein, Crude Fiber, Nitrogen Free Extract, Ether Extract and Total Ash*

INTRODUCTION

Rice polish is derived from the outer layers of the rice caryopsis during milling and consists of pericap, seed coat, nucleus, aleurone layer, germ and part of sub-aleurone layer of starchy endosperm (Juliano, 1988). Rice polish is a byproduct of rice milling industry and is the cheapest source of energy and protein for poultry feeding. It constitutes about 10% of paddy and is available in large quantities in major rice growing areas of the world (Houston and Kohler, 1970).

Rice polish supplies total digestible nutrients almost close to maize (Singh and Panda, 1988). Use of rice polish in poultry industry may reduce feed cost per kilogram weight gain (Khalil et al., 1997a; Shih, 2003). Rice polish is a major cereal by-product available for animal feeding in rice-growing countries. It is a good source of protein (13.2 to 17.1%), fat (14.0 to 22.9%), carbohydrate (16.1%), fiber (9.5 to 13.2%), vitamins and minerals (Vargasgonzalez, 1995; Aljasser and Mustafa, 1996; Ambashankar and Chandrasekaran, 1998).

Nutritive value of rice polish is comparable to other cereals like maize, wheat, and sorghum. It is also a rich source of phosphorus, potassium, iron, copper and zinc, and the amino acid profile of the rice bran protein is generally superior to that of cereal grains. The fiber contents range from 10-15% (Farrell, 1994).

Rice polish has better assortment of amino acids, particularly lysine and methionine, compared to other cereal grains, including corn and wheat (Khalique et al., 2004). In addition to macronutrients, vitamins, minerals, medicinally important antioxidant and γ-oryzanol content of rice polish has recognized it as a potential feed (Iqbal et al., 2005; Moldenhauer et al., 2003; Chatha et al., 2006). Research conducted during the last two decades has shown that rice polish is a unique complex of naturally occurring antioxidant compounds (Iqbal et al., 2005; Moldenhauer et al., 2003).

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Inclusion of rice polish in the diet does not affect the health of chickens (Mahbub et al., 1989). In experiments with chicks, cereal grains have been replaced with rice polish, and it was found promising in certain stitutions (Khalil et al., 1997a).

Despite wide range of advantages, the quality of rice polish available in the local market is questioned. Because, rice husk and saw dusts are frequently incorporated into it to make it cheap. Therefore, the present study was aimed to investigate the chemical composition of rice polish used as poultry feed available in the local market.

MATERIALS AND METHODS

Study area

Livestock and poultry feeds are mostly available in Pahartali, Khatungonja and Karnaphuli markets of Chittagong division. Almost all farmers collect their poultry feeds from these markets Therefore, local markets available in those areas were selected as the study are for collection of sample.

Collection of sample

Samples were collected by using simple random sampling technique. Twenty feed shops were selected randomly. Approximately 500 grams of rice polish was purchased from each shop. Samples were wrapped up by polythene bag and preserved in the laboratory for chemical analysis.

Preparation of sample

Samples were subjected to grinder to make it homogenous powder. Later on, it was mixed properly and exposed to shade to cool down for sampling. Individual samples were identified by marker and subjected to chemical analyses.

Analysis of sample

Chemical analyses of the samples were carried out in triplicate for moisture, DM, CP, CF, NFE, EE and TA in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh as per AOAC (1994).

Calculation of ME

ME was calculated separately for all 20 different rice polish samples. Calculation was performed by mathematical formula as per Ludhi et al. (1976).

Statistical analysis

Data related to chemical composition of rice polish were compiled by using Microsoft Excel 2007. One sample t-test was performed to analyze the data by using Stata 11C. For each t-test, reference value for the relative component was obtained (Banerjee, 1995) to use as the test value for that particular component. Statistical significance was accepted at 5% level ($P<0.05$).

RESULTS AND DISCUSSION

Moisture content did not differ significantly ($P>0.05$). Minimum, maximum and mean values for moisture content were 4.0, 11.4 and 8.0 respectively. In present study, mean value for moisture in rice polish was 8.0 g/100g. The result is in agreement with Banerjee (1995) who found 8.2 g/100g moisture in rice polish. Malik et al. (1979) also obtained 7.4 g/100g moisture in rice polish. Other investigators (Anjum et al., 2007; Hamid el al., 2007; Sharif et al., 2005; Sirikul et al., 2009) also found closely similar results. However, the result of the current study is contradictory with Rao and Reddy (1986) who found 18.10 g/100g moisture in rice polish.

Similar to moisture, DM content did not differ ($P>0.05$). Minimum, maximum and mean values for DM content were 88.6, 96.0 and 92.0 respectively. Mean value for DM in rice polish was 92.0 g/100g. The result is in agreement with Banerjee (1995) who found 91.8 g/100g DM in rice polish. Malik et al. (1979) also obtained 92.6 g/100g DM in another study. Anjum et al. (2007), Hamid el al. (2007), Sharif et al. (2005) and Sirikul et al. (2009) also found similar results. However, the result of the current study is inconsistent with Rao and Reddy (1986) who found 81.9 g/100g DM in rice polish.

Unlike moisture and DM, CP content differed significantly ($P<0.01$). Minimum, maximum and mean values for CP content were 4.7, 14.9 and 8.8 respectively. In present study, mean value for CP was 8.8 g/100g. The result is consistent with other investigators (Anjum et al., 2007; Hamid el al., 2007; Sirikul et al., 2009) also found closely similar results. However, it differs with Rao and Reddy (1986) who found 12.7 g/100g CP in rice polish. Banerjee (1995) found 12.0 g/100g CP and Malik et al. (1979) found 11.45 g/100g CP. Similarly, result of the current study is inconsistent with other investigators (Alencar and Alvarenger, 1991; Gnanasambandam and Hetiarachchy, 1995; Kahlon and Smith, 2004; Saunder, 1990; Sekhon et al., 1997; Sharif et al., 2005; Sikka, 1990).

Similar to CP, CF content differed significantly ($P<0.01$). Minimum, maximum and mean values for CF content were 6.4, 41.5 and 25.2 respectively. In present study, mean value for CF was 25.2 g/100g. The result is contradictory with Rao and Reddy (1986) who found 7.60 g/100g CF in the rice polish. Similarly Banerjee (1995)

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also found 11.2 g/100g CF and Malik et al. (1979) found 3.85 g/100g CF in the rice polish. Other researchers (Gnanasambandam and Hetiarachchy, 1995; Hamid el al., 2007; Saunder, 1990; Sekhon et al., 1997; Sharif et al., 2005; Sikka, 1990; Kahlon and Smith, 2004) also found similar results.

Table 1 - Chemical composition (g/100g) of individual rice polish

Sample No.	Nutritive value (g/100g)							
	Moist.	DM	ME	CP	CF	NFE	EE	Ash
1	11.4	88.6	2562.3	14.5	8.0	52.9	5.0	8.2
2	8.0	92.0	1321.8	6.5	36.0	25.5	4.0	20.0
3	9.6	90.4	3071.1	13.3	8.5	47.0	15.0	6.6
4	8.2	91.8	1496.4	6.3	34.5	31.0	4.0	16.0
5	9.4	90.6	3086.9	13.7	6.4	49.4	14.0	7.1
6	7.6	92.4	1353.1	5.6	40.6	25.1	5.0	16.1
7	10	90.0	2524.4	12.8	12.0	46.7	8.0	10.5
8	10.2	89.8	2516.2	11.9	12.8	47.4	8.0	9.8
9	6.8	93.2	1568.9	6.3	33.5	37.7	2.0	13.7
10	5.2	94.8	1886.2	4.7	30.7	44.4	4.0	11.0
11	9	91.0	1518.8	6.1	31.4	38.6	1.0	13.9
12	7.2	92.8	1542.5	6.3	34.6	32.4	4.0	15.5
13	9.2	90.8	2947.8	12.4	9.2	44.2	15.0	10.0
14	4.8	95.2	1649.6	5.3	34.6	32.2	6.0	17.2
15	5.8	94.2	1801.2	5.2	32.8	39.1	5.0	12.1
16	5.4	94.6	1501.3	6.1	36.8	29.1	5.0	17.6
17	4	96.0	1740.2	5.8	41.5	25.4	10.0	13.3
18	8.6	91.4	2969.3	14.9	10.2	35.6	18.0	12.7
19	9.6	90.4	1664.4	6.7	35.8	31.2	6.0	10.7
20	9.6	90.4	3037.5	10.8	11.6	41.8	18.0	8.2

[DM]Dry matter; [CP]Crude protein, [CF]Crude fibre, [NFE]Nitrogen free extract, [EE]Ether extract

NFE content differed significantly ($P<0.01$). Minimum, maximum and mean values for NFE content were 25.1, 52.9 and 37.8 respectively. The mean value obtained in present study is in agreement with (Anjum et al., 2007; Farrel, 1994; Hamid el al., 2007; Kahlon and Smith, 2004; Sharif et al., 2005).

EE content did not differ significantly ($P>0.05$). Minimum, maximum and mean values for EE content were 1.0, 18.0 and 7.85 respectively. In present study, mean value for EE was 7.85 g/100g. The result is in agreement with Choo and Sadiq (1982) who found 9.5 g/100g EE in rice polish. Similarly, Hamid el al. (2007) also found 8.7-18.9 g/100g EE in rice polish. Anjum et al. (2007) also obtained 9.72 g/100g ether EE in rice polish. However, the result of the current study is contradictory with Banerjee (1995) who found 13.9 g/100g EE in the rice polish. Malik et al. (1979) found 13.65 g/100g EE in rice polish. Findings of other investigators (Kahlon and Smith, 2004; Saunder, 1990; Sharif et al., 2005; Sikka, 1990; Sirikul et al., 2009) are also inconsistent with present study.

Table 2 - Mean values for chemical composition (g/100g) of rice polish

Parameters	Minimum	Maximum	Mean	SD	SE	Sig.
Moisture (g/100g)	4.0	11.4	7.98	2.1	0.46	NS
DM (g/100g)	88.6	96.0	92.0	2.1	0.46	NS
ME (kcal/kg)	1321.8	3086.9	2088.0	661.3	147.9	**
CP (g/100g)	4.7	14.9	8.8	3.7	0.83	**
CF (g/100g)	6.4	41.5	25.2	13.2	2.96	**
NFE (g/100g)	25.1	52.9	37.8	8.6	1.93	**
EE (g/100g)	1.0	18.0	7.85	5.3	1.18	**
Ash (g/100g)	7.1	17.6	12.5	3.7	0.84	NS

[DM]Dry matter; [CP]Crude protein, [CF]Crude fibre, [NFE]Nitrogen free extract, [EE]Ether extract; [SD]Standard deviation, [SE]Standard error; [NS]Non-significant ($P>0.05$); **Significant at 1% level ($P<0.01$)

Ash content did not differ significantly ($P>0.05$). In present study, mean value for Ash was 12.5 g/100g. The result is in agreement with Banerjee (1995) who found 13.6 g/100g ash in rice polish. Similarly Malik et al. (1979) obtained 10.80 g/100g ash in rice polish. Other investigators (Anjum et al., 2007; Gnanasambandam and Hetiarachchy, 1995; Kahlon and Smith, 2004; Saunder, 1990; Sekhon et al., 1997; Sirikul et al., 2009) found similar results. The result of the current study is contradictory with Rao and Reddy (1986) who found 17.4 g/100g ash in rice polish. Similarly Ghazi (1992) also who found 17.15 g/100g ash in rice polish. Anjum et al. (2007) obtained only 5.9 g/100g ash in rice polish.

CONCLUSION

Rice polish is a vital component of the traditional maize soybean based broiler and layer diet. In developing countries, out of all the crop residues, this is one of the cheapest and largest sources of metabolizable energy as

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well as crude protein. There is no doubt that, inclusion of rice polish will substantially minimize cost of production for livestock and poultry. However, current study indicates that the quality of rice polish is widely variable. Therefore, to formulate least cost balanced ration, rice polish must be analyzed first in the laboratory and then incorporate it into the practical ration.

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SUBSTITUTION OF LYSINE WITH MUSHROOM (*Pleurotus cystidiosus*) IN BROILER CHICK'S DIET

C.D. EZEONYEJIAKU[1], C.I. EBENEBE[2], J.J. OKEKE[3], M.O. OBIAKOR[4], C.O. EZENWELU[5]

[1,2,3]Department of Zoology; [4]Department of Environmental Management; [5]Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B., Awka, Anambra State, Nigeria.

*E-mail: maxiugobiks@yahoo.com

ABSTRACT: *Effect of inclusion of mushroom (Pleurotus cystidiosus) to substitute lysine in the diet of broiler chicks was investigated. The study lasted for a period of twelve weeks. Twenty four broiler chicks were subjected to two different dietary treatments (Diet I contained 0.22% of mushroom while Diet II contained 0.22% of synthetic Lysine and was used as control). The different treatments had four replicates of three birds each housed in a metabolic cage. Two parameters, mean weight gain and mean feed intake were recorded. Student t- test showed that there was no significant difference (P>0.05) in the mean weight gain for the chicks on the two treatments (DI-3550g and DII-3375g) and mean feed intake for the chicks on the two treatments (DI-502.5g and DII-420g). Consequently, the observed results showed that mushroom can be used to substitute lysine in the diet of broiler chicks.*

ORIGINAL ARTICLE

Key words: *Mushroom, Lysine, Broiler Chicks, Amino-Acid*

INTRODUCTION

In recent times, cost of chicken has increased due to high price of feed ingredients, the cost of fish meal and soybeans, the two main protein ingredients in poultry feeds has gone up substantially as well as the cost of synthetic amino acid used to make up the protein requirements of monogastrics (Sunil, 2007). The high price of feed ingredients has affected the size and supply of chicken in the market (Kurmanath, 2006). Transportation problems, delivery cost and already high price of feed has put chicken out of the reach of consumers and most of the needed ingredients for chicken are not locally available and have to be imported (Shaiful, 1992). This has necessitated the search for cheap feed ingredients by animal scientists and nutritionists and the research torchlight is now being directed to use of wastes such as sawdust, corncobs and crop straws in poultry feeding. Though monogastrics cannot utilize the cellulose-bound wastes but fungi grown on such wastes become useful feed ingredient for non-ruminant (Fibi, 2007). Wastes like sawdust have caused serious environmental problems like fire explosion in mills and air pollution which results from burning sawdust. Fasidi and Kadiri (1993) reported that utilizing sawdust as compost for growing agricultural products like mushroom can help in ameliorating environmental hazards caused by sawdust.

Mushroom has been found to have some nutritional values that can enhance the growth and performance of broiler chickens and human beings in general. Mushroom contains almost all the essential and non-essential amino acid with lysine as the most essential amino acid (Oei, 2005). Lysine as an amino acid in the diet of chickens and human beings will enhance growth and development. It is also known to contain vitamins (B_1, B_2, and C), carbohydrates, minerals and low fat (Oei, 2005). There were reports on beneficial effects of mushroom, which are used as feed supplements and medicines in chickens (Ogbe et al., 2008; Ogbe et al., 2009). Some medicinal properties have been found in mushroom like antiviral, anti-tumor, immune enhancing and anti-inflammatory, rejuvenating and cholesterol reducing properties (Fasidi, 2006).

In the light of the above extracts on mushroom, the aim of this study is to investigate the nutritive value of mushroom (*Pleurotus cystidiosus)* and to assess the contribution of this mushroom on the performance of broiler chicks if it is used to substitute lysine in their diet.

MATERIALS AND METHODS

Experimental Animals

The animals used for the experiment were twenty-four broiler chicks of two weeks old irrespective of gender. The animals were vaccinated against Newcastle disease and gumboro-disease. They were later treated with Oxytoyin broad spectrum antibiotics and Amprolium to prevent Coccidiosis. The chicks were maintained for two weeks to acclimatize in the new environment before commencement of the experiment.

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Experimental Diet

Twenty-four kilogram of fresh mushroom was used for the study. The mushroom was sun-dried and later milled using hamabill milling machine.

Formulation of Experimental Diet

Two diets, I and II were prepared for the study. In the first diet, 0.02kg of mushroom was used to substitute lysine, while diet II which served as the control contained 0.02kg of lysine without any mushroom. The composition of the diets is as shown in tables I and II.

Table 1 - Diet 1 (Mushroom)

Feed Name	Quantity (Kg)
Mushroom	0.02
Soyabean	2
Maize	5.65
Fish meal	0.4
Dried brewers grain	0.5
Palm oil	0.2
Bone meal	0.25
Oyster shell	0.05
Vitamin/mineral premix	0.05
DL-Methionine	0.03
Salt	0.05

Table 2 - Diet 2 (Control)

Feed Name	Quantity (Kg)
Lysine	0.02
Soyabean	2
Maize	5.65
Fish meal	0.4
Dried brewers grain	0.5
Palm oil	0.2
Bone meal	0.25
Oyster shell	0.05
Vitamin/mineral premix	0.05
DL-Methionine	0.03
Salt	0.05

Data Collection

The feed intakes of the chickens were recorded daily using the formula below;

*Daily feed intake= feed fed – weight of left over

The chicks were also weighed weekly and the record of the weight increase recorded throughout the 12 weeks period of the investigation.

Statistical Analysis

The data collected were subjected to statistical analysis using student's *t*-test to compare means.

RESULTS

The result of proximate composition of the mushroom meal (*Pleurotus cystidiosus)* is shown in table 3 while that of the two experimental diets is as shown in tables 4 and 5 respectively.

Weight changes on the broiler chicks fed on diet I increased from initial weight 2000g to final weight of 4450g, while those fed on diet II increased from initial weight of 2150g to final weight of 4300g at the end of twelve (12) weeks period of the study.

Statistical analysis using student *t*-test showed that there was no significance difference (P>0.05) for the overall feed intake and mean weight gain of the broiler chicks fed on the two different diets.

Result of feed intake and weight changes of chicks in the two dietary groups are presented in tables 6 and 7.

Table 3 - The proximate analysis of the mushroom meal (*Pleurotus cystidiosus*)

Nutrient	% Composition in the Mushroom Meal (*Pleurotus cystidiosus*)
Crude protein	25.0
Carbohydrate	58.0
Moisture	91.5
Ash	9.3
Fat	1.6
Fibre	11.5
Energy (Kcal dry maternal)	265

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Table 4 - The Proximate analysis of the experimental diet I with mushroom (*Pleurotus cystidiosus*)	
Nutrient	**% Composition in the Experimental Diet I**
Crude protein	20.78
Fat	3.82
Dry matter	89.46
Crude fibre	2.03
Ash	4.98
Moisture	10.54
Nitrogen free extract	57.85

Table 5 - The Proximate analysis of the experimental diet II with Lysine	
Nutrient	**% Composition in the Experimental Diet II**
Moisture	9.76
Dry matter	90.24
Crude protein	22.05
Crude fibre	1.8
Fat	2.52
Ash	5.59
Nitrogen free extract	58.33

Table 6 - Weekly records of feed intake for two dietary groups (g)

Dietary	W 1	W 2	W 3	W 4	W 5	W 6	W 7	W 8	W 9	W 10	W 11	W 12	Total	Total Mean
Diet I	390	640	420	560	390	420	560	640	425	385	640	560	6030	502.5
Diet II	400	370	460	450	370	460	400	450	460	370	400	450	5040	420

W=week

Table 7 - Weekly changes in weight gain of the broiler chicks feed on two different Diets

Dietary	Initial Wt	W 1 Wt	W 2 Wt	W 3 Wt	W 4 Wt	W 5 Wt	W 6 Wt	W 7 Wt	W 8 Wt	W 9 Wt	W 10 Wt	W 11 Wt	W 12 Wt	Total	Total Mean
Diet I	2000	2350	3100	3,100	3200	3,300	3300	3600	3600	4100	4200	4300	4450	42600	3550
Diet II	2,150	2400	2800	3050	3,300	3,300	3,350	3400	3450	3450	3750	3950	4300	40,500	3,375

W=week, Wt= weight

DISCUSSION

The study showed that there was no significant difference in the feed intake of broiler chickens fed on the two diets and their weight gains. This may be attributed to the high lysine content of mushroom as reported by Oei (2005) that mushroom contained all the essential and non-essential amino acids with lysine as the most abundant essential one. But the numerical variation between the two values in the weight of the two experimental subjects could also be attested to the similar high nutritional contents of the mushroom. Alternatively, there might be difference in the feed conversion process of the two diets among the broilers and possibly their gender, age and duration of feeding. The disparity in age, gender, duration, and method of feeding is documented in a report by Tolkamp (2005).

The current study is consistent with the report of Fasidi (2006) that mushroom protein and amino acid can compete with similar nutritional elements from any other source. This is further supported by the work of Guo et al. (2004) that showed increase BW (Body weight) gain in broilers with the use of mushroom and herb polysaccharides. Consequently, going by the report of Oei (2005), mushroom is a healthy diet since it contains good amount of protein and amino acid, supporting the present investigative report and scientific claims. The medicinal effects and values have been earlier documented by Ogbe et al. (2008) and Ogbe et al. (2009).

CONCLUSION

Mushroom meal (*Pleurotus cystidiosus*) has been proved to be of high nutritional value in terms of its effect on the growth and performance of broiler chicks. The contents of amino acid in mushroom especially lysine can comfortably match any synthetic amino acid (lysine) as carried out in this study that can help in the growth and performance of broiler chicks. In this era of high price of feed ingredients which has denied majority of Nigerians animal protein from chicken, cultivating of mushroom and using it to substitute lysine in feed formulation for broiler chicks will go a long way in enhancing availability and food security of chickens with affordable market price.

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EVALUATION OF I_2 THERMOSTABLE NEWCASTLE DISEASE VACCINE ON LOCAL CHICKENS IN SELECTED DISTRICTS OF WESTERN AMHARA

M. NEGA[1]*, F. MOGES[1], H. MAZENGIA[2], G. ZELEKE[1], S. TAMIR[1]

[1]*Andassa Livestock Research Center P.O.Box 27 Bahir Dar, Ethiopia*

[2]*Bahir Dar University, College of Agriculture and Environmental Sciences, P.o.Box:79, Bahir dar, Ethiopia*

*Email: mohammed.nega@yahoo.com

ABSTRACT: *Evaluation of I_2 thermostable Newcastle disease vaccine was conducted in three districts of four local chicken ecotypes using survey and sera analysis from 2010 to 2011. According to the survey result conducted on 160 chicken owners, the major chicken production constraint 77.5% of the area was disease and mortality of chickens by any cause from day old to adult chicken age was 44.6%, ranging from 39.9%, 45.3%, 45.8%, and 46.3% at Melohamusit, Mecha, Farta, and Tillili, respectively from which disease related mortality was 77%. Mortality of chickens due to disease outbreak was usually higher during the beginning of the rainy season, mainly in April (43.1%), May (38.8%) and June (63.8) as first, second and third priority months, respectively and there is significant deference in disease occurrence among seasons. The overall seroprevalence of Newcastle disease in village chickens using Hemagglutination inhibition test (≥1:16) was 55.8%. However, the antibody titer response to I_2 thermostable vaccine was 90.4% ranging from 83.8%, 90.9%, 91.7%, 95.1% in Mecha, Tillili, Farta and Melohamusit, respectively after one vaccination and 93% ranging from 90.9%, 93.3%, 93.8%, 96%, in Mecha, Melohamusit, Tillili and Farta, respectively after booster dose vaccination. There was no significant difference in antibody titer detected between local chicken ecotypes and/ or districts before and after vaccination. However, there was significant difference in antibody titer after 1st (P =0.000) and booster dose (P =0.000) vaccination. A quick survey conducted after the last vaccination showed that mortality of chickens became 8.2% which is reduced by 82% than the mortality before vaccination. In conclusion this vaccine was found very appropriate and effective in reducing village chicken mortality and morbidity, so controlling of Newcastle disease using I_2 thermostable vaccine could be a key to the development of village chicken production.*

Key words: *Hemagglutination, I_2 thermostable vaccine, Newcastle disease, Village chickens*

ORIGINAL ARTICLE

INTRODUCTION

In Ethiopia, village chickens have been reared for a long time for different purposes in addition to meat and egg production. They have a big contribution to the country's economy. This is not because they are productive but are huge in number Alemargot (1987). According to many studies constraints which restrict the potential of village chickens in Ethiopia include; the presence of diseases of various natures, low inputs of feeding, poor management, and lack of appropriate selection and breeding practices (Alemu, 1995; Ashenafi, 2000; Tadelle and Ogle, 2001).

Newcastle disease (NCD) is among the major constraint to production of village chickens in many developing countries (Spradbrow, 1988; Alexander, 2001). It is the most important viral disease recognized in tropical countries in village poultry production systems. The disease causes great losses in most scavenger and commercial flocks (Spradbrow, 1988; Alders, 2001). Recently, the highly infectious ND is reported to have almost reached 100% mortality in some African countries (Kitalyi, 1997; Tadelle and Ogle, 2001; Tadelle and Jobre, 2004; Mazengia et al., 2009).

Newcastle disease (NCD) is a highly contagious viral disease that attacks many species of domestic and wild birds Al-Garib et al. (2003). The causal agent is the Newcastle disease virus (NCDV) which is a negative sense single stranded RNA virus belonging to the family *paramyxoviridae.* The strains of Newcastle disease virus are classified into highly virulent (velogenic), intermediate (mesogenic), or avirulent (lentogenic) based on their pathogenicity in chickens Beard and Hanson (1984). NCDV infections of poultry range from latent to rapidly fatal depending upon the pathotype of virus involved Alexander (2003). The transmission of NCDV occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment and clothing Tu et al. (1998).

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NCD is mentioned as one of the disease problems in farms and backyard chickens in most parts of Ethiopia. It has many different local names in different areas and the most common one is "Fengil'" (Nasser, 1998; Ashenafi, 2000; Tadelle and Ogle, 2001), which means sudden dorsal prostration and signifies the acuteness and severity of the disease. It is possible to say that currently there are no low risk areas for NCD remaining in Ethiopia. The disease has already become endemic in village poultry population and thus it recurs every year inflicting heavy losses (Tadelle and Jobre, 2004; Mazengia et al., 2009). It is estimated that annual outbreaks of NCD kill 70–80% of unvaccinated village chickens Spradbrow (1995). Outbreaks are unpredictable and discourage villagers from paying proper attention to the husbandry and welfare of their chickens.

Vaccination is the most important method of disease control particularly to decrease mortality from NCD. Vaccination results in a quite significant increase in chick survival from 30% to 60% Udo (1997). Conventional vaccines are unsuitable for sustained use in village chicken production system because of their cost, large dose presentation and thermolability. The importance of village chickens to the rural and peri-urban poor in developing countries is not contested. Another universal truth is that these flocks are less productive, and cost-effective remedies should be available. Thermostable/heat stable/ Newcastle disease vaccines, suitably applied, have proved effective in many trials under laboratory conditions and in villages Spradbrow (2011).

So the objective of this study is to introduce I_2 thermostable vaccines in village chickens and evaluate the effectiveness of I_2 thermostable Newcastle disease vaccine and to reduce the mortality of village chickens due to Newcastle disease and to increase the awareness of households in particular women about NCD and the control options.

MATERIALS AND METHODS

Study areas

These studies were conducted at three districts (Tillili /Guagusa-Shikudad/, Mecha and Farta districts), located in the North Western part of the country. Local chicken ecotypes collected from these study districts showed relatively better egg and meat production potential when managed intensively at Andassa Livestock Research Center and were recommended for further improvement by Halima (2007). Therefore these districts were selected purposively.

Questionnaire survey

A questionnaire survey was conducted on 160 respondents before the beginning of vaccination to assess the prevailing chicken production system of village chickens and major constraints of the system in selected districts. And after the fifth vaccination a quick survey was conducted on 122 respondents to assess the effect of I_2 thermostable vaccine in reducing the morbidity and mortality of local chickens of the study area.

Vaccination of animals

Before the beginning of vaccination Couple training was given on poultry disease and health management to farmers of the area who have an experience of rearing poultry. After the training vaccination site were selected and practical training were given to selected farmers or community vaccinators on how to vaccinate chickens and how to handle the vaccine.

Chickens were vaccinated with I_2 thermostable Newcastle disease vaccine produced by National Veterinary Institute (NVI), Debre zeit Ethiopia. The vaccines were administered once, every 3 months. Vaccination was given to the whole chicken population in selected villages by community vaccinators/selected farmer/.

Vaccines were diluted using the formula:-

$$\text{Amount of water required in ml} = \frac{\text{1ml X number of doses that the vial contain}}{\text{Number of drops per 1ml of the dropper/ syringe}}$$

Vaccination was given through ocular route and the vaccine costs only 0.15 Ethiopian cents per head/bird. Administration of the eye drop to the bird was done with the dropper in a vertical position to make sure that drops of a uniform size are produced. Chickens were vaccinated five times.

Serum collection

About 2-3ml blood was collected once before vaccination and twice after 3 weeks of each vaccination regime from the wing vein of chicks of all age groups with 5ml disposable syringe/ non-heparinized vaccutainer tube of 5ml and 23G (32mm) needle. The syringe/ tube containing the blood was kept at room temperature overnight in slanting position until the blood clot and the blood was centrifuged with hematocrite centrifuge, then the serum was transferred into a sterile plain tube. The tubes were labeled and stored at -20^{0}C until analysis.

Hemagglutination-Inhibition (HAI)

HAI test was done according to the procedures of OIE (2004). The test was conducted at the National Veterinary Institute (NVI), Debre zeit-Ethiopia. The test was carried out by running two fold dilutions of equal volumes (25µl) of Phosphate Buffered Saline (PBS) and test serum (25µl) in U-bottomed micro titer plates. 4 Hemagglutination units of (HAU) the viral antigen of LaSota (I_2) strain obtained from France was added to each well and the plates were left at room temperature for a minimum of 30 minutes. Finally 25µl of 1% (v/v) chicken RBCs collected from four chickens older than 3-weeks and serologically negative to NCD was added to each well and, after gentle mixing, the RBCs were allowed to settle for about 40 minutes at room temperature. The HI titer was read from the highest dilution of serum causing complete inhibition of 4 HAU of antigen. Those wells that showed sedimentation of RBC as the control wells

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(containing only 25µl RBCs and 5µl PBS) were considered as inhibition. A titer greater than or equal to 1:16 was taken as positive.

Data management and statistical analysis

Basic data entry and handling were done using SPSS software version 16. Descriptive statistics and chi-square tests were employed to summarize the data. Tests were considered significant at $p < 0.05$.

RESULTS

According to the survey result the prevailing chicken production constraint of the area was disease (77.5%) (Table 1), predator (80.6%) and Feed shortage (82.5%) as first, second and third priority problem, respectively. The average mortality rate of chickens by any cause from day old to adult age was 44.6%, ranging from 39.9%, 45.3%, 45.8%, and 46.3% at Melohamusit, Mecha, Farta, and Tillili, respectively from which disease related mortality was 77%. Most of respondents witness high occurrence of chicken diseases, among which 93% of them says Newcastle disease locally known as "Fengil" or "Meyaz", was the major and economically important constraint for the existing chicken production system of the study district. According to interviewed chicken owners, mortality of birds due to disease outbreaks was usually higher during the beginning of the rainy season, mainly in April (43.1%), May (38.8%) and June (63.8) as first, second and third priority months, respectively (Table 2). There is significant difference in disease occurrence among seasons ($P = 0.000$, $df = 7$, $x^2 = 189.1$).

The overall seroprevalence of Newcastle disease in village chickens using Hemagglutination inhibition test (≥1:16) was 55.8% (Table 3). However, the antibody titer of Newcastle disease in response to I_2 thermostable vaccine was 90.4% (255/282) and 93% (265/285) after 1st vaccination and booster dose vaccination respectively (Table 4 and figure 1).

Table 1 - First priority village chicken production constraints in the study district

	Tillili		Mecha		Farta		Total	
Constraints	**Frequency**	**%**	**Frequency**	**%**	**Frequency**	**%**	**Frequency**	**%**
Disease	29	72.5	39	97.5	56	93.3	124	77.5
Feed shortage	2	5.0	0	0.0	2	3.3	4	2.5
Predator problem	7	17.5	1	2.5	20	33.3	28	17.5
Market Problem	0	0.0	0	0.0	2	3.3	2	1.3
Lack of land	1	2.5	0	0.0	0	0.0	1	0.6
Lack of capital	1	2.5	0	0.0	0	0.0	1	0.6

Table 2 - Major seasons of the year when Newcastle disease appear as an outbreak

Months	Frequency	Percent	Valid Percent
March	15	9.4	9.4
April	69	43.1	43.1%
May	17	10.6	10.6
June	39	24.4	24.4
July	13	8.1	8.1
August	2	1.2	1.2
October	2	1.2	1.2
September	3	1.9	1.9
Total	160	100.0	100.0

Table 3 - Status of seroprevalence of Newcastle disease before vaccination

Local chicken ecotypes	Seroprevalence of Newcastle disease	
	Positive (≥1:16)	Negative (<1:16)
	N (%)	N (%)
Tillili	20 (50%)	20 (50%)
Mecha	34 (53.1%)	30 (46.9%)
Farta	33 (60%)	22 (40%)
Melohamusit	34 (58.6%)	24 (41.4%)
Total	121 (55.8%)	96 (44.2%)

Table 4 - Antibody titer of Newcastle disease in four local chicken ecotypes in response to the first vaccination

Local chicken ecotypes	Antibody titer after first vaccination	
	Positive (≥1:16) n (%)	Negative (<1:16) n (%)
Tillili	50 (90.9%)	5 (9.1%)
Mecha	62 (83.8%)	12 (16.2%)
Farta	66 (91.7%)	6 (8.3%)
Melohamusit	77 (95.1%)	4 (4.9%)
Total	255 (90.4%)	27 (9.6%)

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There was no significant difference in antibody titer detected between local chicken ecotypes and/or districts before vaccination (P >0.05, df = 3, x^2 = 1.3), after first vaccination (P > 0.05, df = 3, x^2 = 5.9) and after booster dose vaccination (P>0.05, df = 3, x^2= 1.5). But There was significant difference in antibody titer after 1st (P=0.000, df = 1, x^2=184.3) and booster dose vaccination (P = 0.000, df = 1, x^2=210.6).

In addition, according to a quick survey after vaccination the mean morbidity and mortality of chickens was 13.9% and 8.2% respectively, this shows that mortality of chickens was reduced by 82% as compared to the mortality of chickens before vaccination.

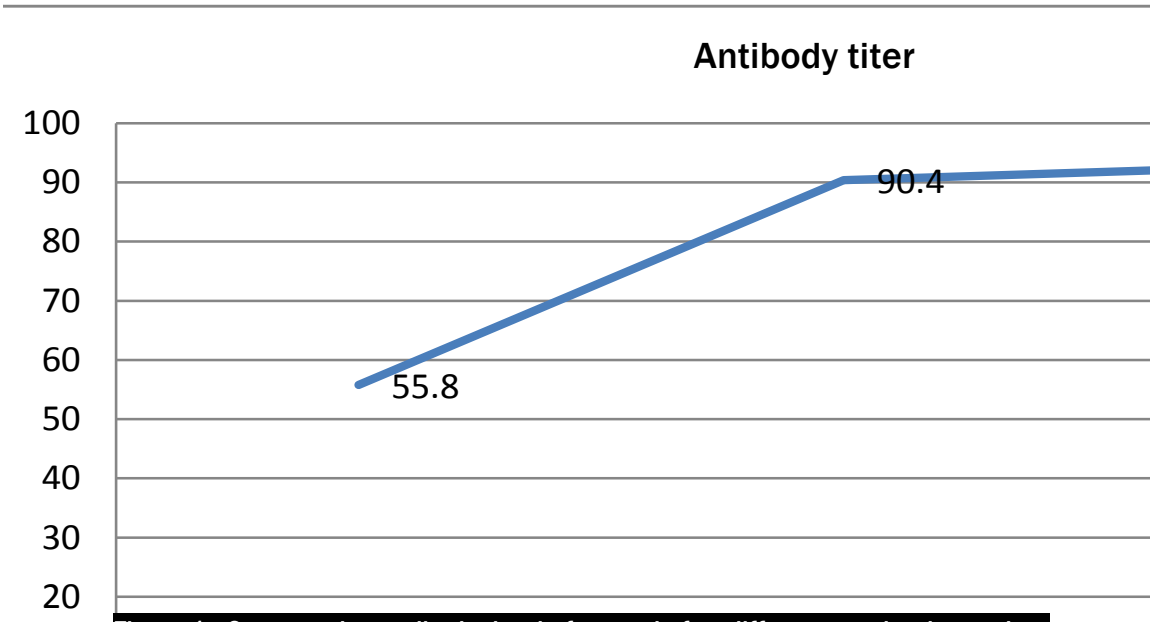

Figure 1 - Comparative antibody titer before and after different vaccination regime

DISCUSSION

The mortality rate of chicken in this study from day-old to adult chicken age was (44.6%), from which disease related mortality is 77%. This finding is in line with the previous reports from both Ethiopia (Alemargot, 1987; Mazengia and Eshetie, 2008) and other countries Farooq (2001) the current disease related mortality from day old to adult chicken age is estimated between 20% and 80%.

On the other hand, the overall seroprevalence of Newcastle disease in village chickens in this study was 55.8%. This finding is in line with the previous reports in Ethiopia by Mazengia et al. (2010). However, this finding is higher than the previous reports in central high lands of Ethiopia by Ashenafi (2000) and the reports of Zeleke et al. (2005) in Rift valley areas of Ethiopia. Similarly higher seroprevalence Newcastle disease was reported by Ezeokoli et al. (1984) who recorded 62.9% seroprevalence in Nigeria.

The overall population with protective antibody titer (≥1:16) after first and booster dose vaccination was 90.4% and 93% in the study districts, respectively. Which is higher than the reports by Mazengia et al. (2009) in day old-chicks in which the overall population with protective antibody titer (≥1:8) was (71.1%) in the study districts, this could be I_2 thermostable vaccine may have a higher capacity of inducing antibody production than the conventional vaccines, or may also be due to challenges in keeping the cold chains of conventional vaccines during vaccination. And this finding is concurrent with the epidemic theory which suggests that if 70% of the population is immune, the disease outbreak is unlikely to occur because there are not enough susceptible to propagate an epidemic (Thrusfield, 1995; Young et al., 2001).

CONCLUSION AND RECOMMENDATION

The major poultry production constraint and causes of mortality in the study area was Newcastle disease locally known as “Fengil” which mostly occurs as an outbreak during the beginning of the rainy season in April and may. I_2 thermostable vaccine have similar response for all type of local chicken Ecotypes and can reach a protective level at one vaccination regime without the need for booster dose vaccination. Despite chickens were vaccinated and vaccines were handled by community vaccinators, I_2 thermostable vaccine is highly suitable and effective in reducing village chicken mortality and morbidity and control of Newcastle disease using I_2 thermostable vaccine is the key to the development of village chicken production. Wider use of this vaccine needs further training of farmers and the adoption of suitable extension methods.

So, emphasis should be given on extensive use of I_2 thermostable vaccine in village chickens in reducing the mortality and improving their productivity and Vaccination programs should be continual and sustainable but if it is not possible chickens should be vaccinated at least once every year before April which may reduce heavy chicken losses. Wider use of this vaccine should be practiced through establishment of community vaccinators and further training of farmers.

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SOME BEHAVIORAL TRAITS OF RED NECK OSTRICH UNDER CAPTIVE CONDITIONS

F.A. MOHAMMED AHMED[1]*, R.R. MOHAMMED SALIH[2]

[1]*Department of Fisheries and Wildlife Science College of Animal Production Science and Technology, Sudan University of Science and Technology P.O.BOX204,Khartoum North, Sudan*
[2]*Department of Clinical Medicine College of Veterinary Medicine University of Khartoum P.O. Box 32, Khartoum North, Sudan*

*Email: fawziali38@yahoo.com

ABSTRACT: *The present study has been conducted to observe some behavioral traits of ostrich under captive conditions. The observations have been carried during the period 14 June to 24 June, 2005, for 8 equal time period, extending for 24 hours from 0600 p.m hour to 0600 p.m hour next day. The bird flack consisted of two adult males and adult female, kept in the Collage farm, in a cage joined to a fence to allow for free movement. The recorded behavioral activities included: standing in the sun, standing in shade, laying in the shade, laying in the sun, staying in the cage, movement and sitting on the knees, feeding, drinking, quarrel, urination, defecation, ritual display, courtship, and preening. It was noticed that the most time consuming activities were standing in the sun, standing in the shade, laying in the shade, and movement. The longest period of the time budget was taken in laying in shade (250.3 min.). The shortest fraction of the time budget was spent in courtship maneuvers (3.25 min.). The main target of the study was to provide ostrich breeders with useful information for better management.*

ORIGINAL ARTICLE

Key words: *Behaviour, Ostrich, Captivity Condition, Birds*

INTRODUCTION

Ostrich (*Struthio camelus camelus*) is the largest and heaviest living bird and is the only bird with just two toes and sole representatives of the order Struthioniformes (Alden et al., 1996). Ostriches produce red meat that is very similar in taste and texture to veal and beef (Du Preez, 1991: Anonymous, 1994). The meat has been reported to be of high protein, and low cholesterol than any other protein of animal origin (Shanawany, 1996). With increasing incidences of human´s heart associated problems in developing countries such as Sudan, ostrich farming would be of importance not only in supplementing protein requirements of the growing population in the country, but also in providing abundant and cheaper supply of meat for people with specific nutritional requirements such as low cholesterol and low fat. Studies on behaviour are important for evaluation of the welfare of animals. Ostriches have not been selectively bred with the objective of minimizing the effect of the stress experienced on farms.

The African ostrich is a social species; therefore it thrives better in groups (Bolwig, 1973). Thus, providing conditions where the animals can express their normal behaviours is one of the basic requirements of good welfare. According to Newberry et al. (2007), in the wild, pecking is a natural behaviour that leads to the establishment of a pecking order in the group. Mitchell (1960) (quoted by Bertram, 1992) published a list of 300 citations dealing with this species, but most of them were devoted to physiology, veterinary aspects, husbandry and marketing, while not involving behavior this was described in detail only in recent decades, firstly by Sauer and Sauer (1966) and Bertram (1992). Even less attention has been paid to a detailed quantitative description of behaviours or behavioural patterns displayed by captive ostriches. In fact, most authors either considered gross daily temporal budgets for a few behaviours (Degen et al., 1989) or made more detailed behaviour descriptions in various contexts, but still in terms of just percentage of time (McKeegan and Deeming, 1997; Deeming, 1997, 1998). As far as this study concern of, there are no studies devoted to the analysis of behaviour in terms of the sequences of behaviour forming the whole behaviour repertoire. Recent increases in ostrich breeding, principally in South Africa and later in Israel and Europe, led to the necessity to learn more about its behaviour in restricted areas.

The study of behaviour transitions helps understanding the sequences of behaviour. It is believed that analysis allows understanding the relationships among different behaviours and their relative importance more deeply than simply ascertaining the duration of each behavior, considered separately from the others (Cronin, 1985; Csermely, 1994).

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The objective of the research work was to study some behavioral traits of Red Neck Ostrich under captive conditions.

MATERIALS AND METHODS

This study was carried out at the farm of College of Veterinary Medicine and Animal Production Sudan University of Science and Technology. Three individuals of Red neck 4-years old ostriches (S. c.camelcus) two male and one female were included in the study. They had been living in unit for at least 3 years.

The part of unit used for the study consisted of two rows of three identical outdoor paddocks, separated by an inspection corridor 4 meter wide. The paddocks were 60 m _ 41 m (1504 m2) in size and delimited by 4 m high wire mesh. Each paddock had a wooden shelter of about 8 m2 (three sides and roof), and contained a trough located at the middle of the paddock's short side in front of the inspection corridor and a plastic water container (80 cm _ 40 cm _ 45 cm) on the ground just outside the shelter.

Two to five small trees (4–5 m high) were scattered in each paddock. The ground was natural, but the grass had been removed by ostrich locomotion and the ground became muddy after rains. Behavioral activities which were observed and recorded included: standing in the sun, standing in shade, laying in the shade, laying in the sun, staying in the cage, movement and sitting on the knees, feeding, drinking, quarrel, urination, defecation, ritual display, courtship, and preening. Simple statistical analysis was performed with the mean and standard divination (Steel and Torrie, 1980).

RESULTS

As shown in Figure 1, 2 and 3. The total observation duration for the three individual as a whole was 240 hours. Males and females were recorded equally. Both sexes were mostly involved in similar activities. Closed-wing Walking and Environment Pecking. These behaviors took up 39.0% and 41.1% of time in males, respectively, and 40.4% and 43.1% in females, respectively. Walking, as a whole, plus running, as a whole, involved almost one half of observation time in both sexes (13.2%). Birds also spent 11.9% of time in standing in the sun, 1.6% in eating and 0.5% in raised-head resting. On the other hand, ostrich were involved for 25.9% of time in standing in the shade, 28.8% of time in laying in the shade and 5.3% of time in staying in the cage.

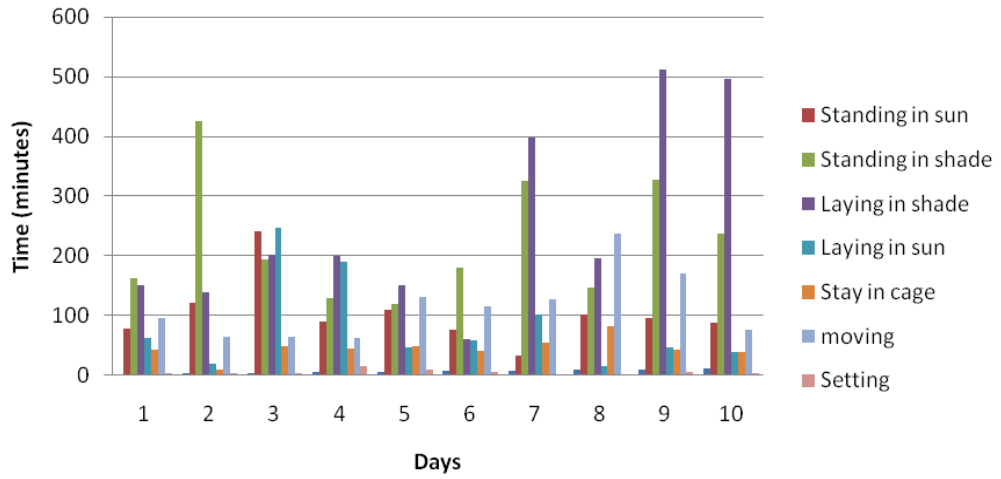

Figure 1 - The duration in minutes of some behaviors displayed by ostriches in 24 hours for 10 days

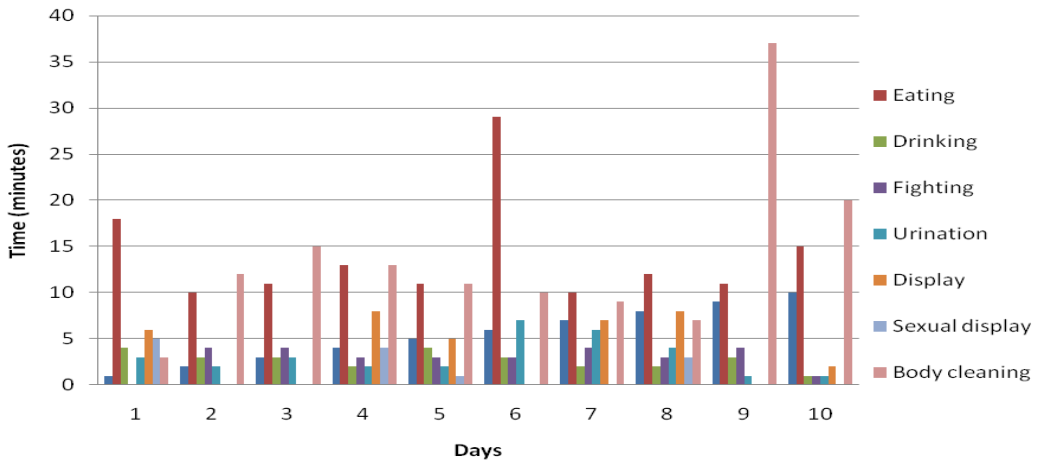

Figure 2 - The duration in minutes of the behaviors displayed by ostriches in 24 hours for 10 days

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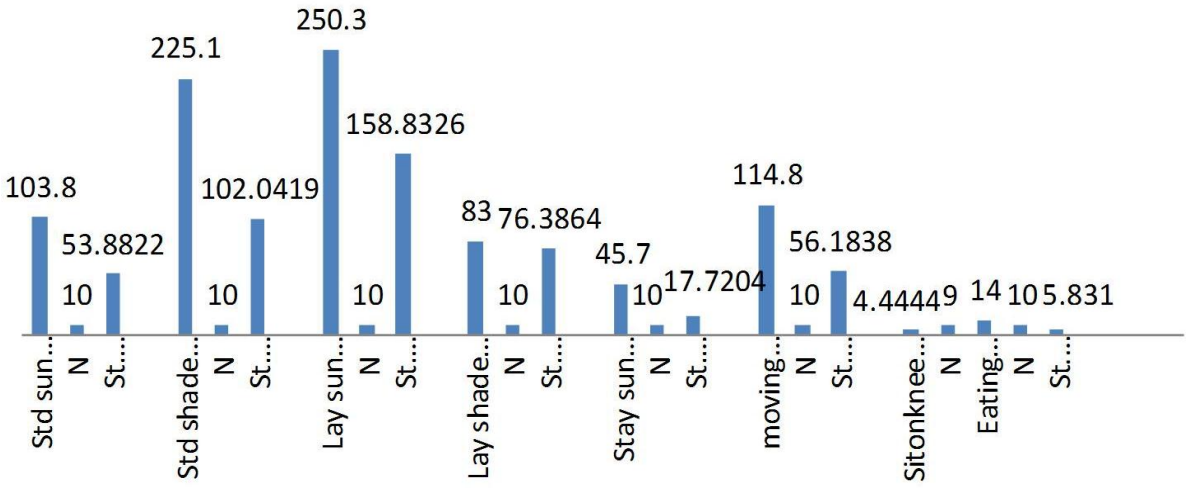

Figure 3 - The high frequency behaviors recorded among various ostrich. (Std: Standing. St: Standard. N: Normality)

DISCUSSION

The proportion in the time–activity budgets displayed by the male and female ostriches are largely comparable with data recorded by others in both Europe (Sambraus, 1994; Milton and Dean, 1995; Ross and Deeming, 1998) and South Africa (Lambrechts et al., 1998). This primarily concerns walking in general, comprising the several variations considered, but also other important behaviours such as standing and eating, the present study showed the same behaviour types dominating the activity budgets recorded by other authors. There are some discrepancies in the values, probably due to the different recording methodology used to record of the behaviour. Although sexual differences were beyond the aims of this work, marked sexual differences in percentage time–activity budgets do not seem to exist, as instead reported by McKeegan and Deeming (1997) and Lambrechts et al. (1998), but this might be due to the longitudinal nature of the present study, which could increase overall similarity of activity in both sexes. The great majority of the recorded behavioural transitions were one-way transitions in both sexes and age.

The time–activity budgets showed fact that there was some sort of variability among the behaviours. Some of them, e.g. walking and pecking the environment, have great incidence in the repertoire of wild ostriches as well. The ostriches stood still more in the morning than in the middle of the day. The birds also walked less in the middle of the day. They ran and drank more in the afternoon that at noon. The lowest frequency of eating and lithophagia were found at noon. Coprophagia and dancing were less observed in the middle of the day than in the afternoon. Dust-bath was observed mostly in the afternoon. High temperatures of approximately 35°C from 11:00 to 3:00 p.m. during experimental period may have caused a reduction in bird activity during this period. The birds were observed drinking water and feeding with lower frequency in the middle of the day than to the other periods.

Both drinking and feeding were different to period 3. According to Souza (2004), ostriches in captivity and in hot weather conditions drink water at dawn and at dusk. During the trial period, there was a higher consumption of feed in the early hours of the day and during the afternoon, which coincides with the hours of lower environmental temperature, as well as the time when food is distributed. Nevertheless, higher temperatures from 11:00 a.m. to 3:00 p.m. may have led to a decrease in the food consumption to avoid caloric enhancement resulting from the digestion process. Sambraus (1994) and Deeming (1998) also found higher consumption of food during the morning when the food was supplied also McKeegan and Deeming (1997), who observed pairs of adult ostriches in captivity, reported a peak in the consumption of feed in the morning for both sexes.

In the present study, temperature and food offered were confounded, therefore it was not possible to separate their effects. On the other hand, it is reported by Sauer and Sauer (1966) that ostriches in natural environment spend most of the time walking and feeding during the day, including grazing and seeking other kinds of food. This probably relates exploratory behaviour with the aim of achieving the daily nutritional requirements. In this study, the ostriches at the hours close to noon, showed a calm behaviour. They spent more time standing in the early hours of the day. Deeming (1998) found no diurnal variation in the frequency of this behaviour during the winter in captive ostriches. Similar results were found by Ross and Deeming (1998), who studied the behaviour of ostriches during the summer. In this study, the birds were moving more in the early hours of the day and at the end of the afternoon, which is in accordance with the results found by McKeegan and Deeming (1997), who reported greater expression of this behaviour in the morning and afternoon in captive male ostriches.

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HATCHABILITY OF GUINEA FOWLS EGGS AND PERFORMANCE OF KEETS UNDER THE TRADITIONAL EXTENSIVE SYSTEM IN TOLON-KUMBUNGU DISTRICT OF GHANA

J. NAANDAM*, G.B. ISSAH

Department of Animal Science, Faculty of Agriculture, University for Development Studies, Tamale, Ghana

*Email: jaknaan@yahoo.com

ABSTRACT: *A study was carried out to examine the hatchability of guinea fowls eggs and performance of keets under the traditional extensive system. A short questionnaire to ascertain production scope and management practices were administered to a total of ten farmers; five farmers from each of two communities, using purposive sampling. In order to establish some actual production indices, data was collected from the sampled farmers on mean number of eggs incubated, mean weight of eggs incubated, mean number of eggs hatched, percentage hatchability of eggs, mean weekly numbers of keets, mean weekly weight gain of keets, total weight gain of keets and mortality rate of keets. Data were analyzed using Genstats Discovery (3rd edition) and SPSS version 17. The main breeds of guinea fowls kept by farmers were the pearl and the lavender. The methods of identifying fertile eggs by farmers were by the use of size and texture of eggs. Majority of the farmers (80%) fed their guinea fowls with maize, while (20%) fed them with millet before egg laying, but during egg lay 80% of the farmers fed their guinea fowls with millet for the reason that it increased egg production. For the production indices, there were significant differences (P<0.001) in mean weekly numbers of keets and mean weekly weight gain of keets for the study period. A much lower significant difference (P<0.05) was observed for the total weight gain of keets, possibly because weight gain through growth over stripped the weight losses through mortality. Mortality rate of keets was high ranging between 61-69% within the two communities, though these did not significantly differ from each other. Mean number of eggs incubated was 18.4 for Nafaring community and 25.4 for Cheyohi community. Similarly the mean weight of eggs incubated, total weight of eggs incubated, number of eggs hatched and percentage hatchability (%) were 31.4g and 31.8g, 577.8g and 807.7g, 13.4 and 18.6, 72.8% and 73.6%, respectively. There were significant differences in performance indices across the weeks but not between the two communities.*

Key words: *Communities, Hatchability, Keet Performance Traditional Extensive System, Mortality*

ORIGINAL ARTICLE

INTRODUCTION

Guinea fowl production provides cash for investment in crop and livestock production (Karbo and Bruce, 2000). The meat and eggs from guinea fowl provide a good source of protein for rural folk, which can be used to balance the inadequate intake (Smith, 1990). Guinea fowl and its products are given to very important visitors like in-laws and part-payment of dowries in most parts of northern Ghana. Also the feathers of guinea fowl are used in making pillows and for aesthetic purposes in homes, restaurants and hotels. The local cracking call of guinea fowl especially when they see strange objects in the vicinity makes them potential guards. They are therefore kept as feathered "watchdog" which can protect poultry and alerting people (Managa and Haule, 1994).

There is some evidence to suggest that over 50% of the rural folks do not depend on industrially produced poultry and its products (Reddy and Qudratullah, 1996; Shitu, 2003). Hence attention is now being focused on poultry production at the village level in order to boost output. The local guinea fowl is one of the poultry species receiving more attention in northern Ghana because of its significant role in the lives of the people.

The production of guinea fowl in the rural and traditional system of management is faced with many problems including: diseases and internal parasites, inadequate feeding, unavailability of eggs for hatching in the dry season, low growth rate and lack of improved genetic materials (Okaeme, 1984; Ayorinde, 1989; Nwagu and Alawa, 1995; Idi., 1997; Karbo et al., 2002; Tanko, 2003), resulting in low production. Paucity of information on traditional guinea fowl production is hampering the development of this industry. As a result, the potential of the guinea fowl industry has remained rudimentary and undeveloped for long a time (Karbo et al., 2002). It is against

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this background that this study set out to assess egg lay, hatchability and fertility of guinea fowl eggs and also to determine performance of keets under the traditional extensive system.

MATERIAL AND METHODS

Study area

The study was conducted in two communities, Nafaring and Cheyohi, near the University for Development Studies, Nyankpala. These two communities are about 8 km apart. Nyankpala is approximately 16km West of Tamale. It has unimodal rainfall pattern. The area lies on latitude 09°25"N and longitude 00°58"W with an altitude of 183m above sea level. The mean annual rainfall and temperature are 1043mm and 28.3°C respectively. The rainy season is usually between April and October with the dry season from November to March. The mean annual day time humidity is 54% (Kasei, 1990).

Sampling procedure

The two communities were sampled because they were known to have good numbers of guinea fowl farmers and also because of convenience of accessibility for the researchers. Purposive sampling was used to locate guinea fowl farmers and within these farmers who were willing to take part in the monitoring of their flocks, simple random sampling was used to gather the required information.

Duration

The monitoring and collection of data on incubated eggs, hatchability and the performance of keets up to the fifth week was commenced in September 2010 to October 2010. The semi-structured questionnaire was administered alongside as and when farmers were visited.

Data collection

Data on breed type kept, feed type used, number of eggs laid/bird/year, feed type used and fertility detection method in eggs was collected using semi-structured questionnaire that was administered to ten respondents in two communities; five from each community. Additionally respondents were visited for about 6 times and data on the following production indices were monitored and collected for 4 consecutive weeks starting from the second week as it was not possible to be present at hatching or the day after, as follows:

Weight of eggs: The eggs were weighed before incubation using an electronic weighing scale. The eggs were put in badges and after which the weight was calculated. A perforated box was kept on the weighing scale and adjusted to zero before the eggs were kept into the box and the reading noted.

Weight of keets: The keets were weighed weekly early in the morning before feeding using an electronic weighing scale. The keets were weighed in badges after which the average weights were calculated. A perforated box was again kept on the weighing scale and adjusted to zero before keets were kept into the box and the weight recorded. The weekly weight gain was then calculated from the weekly weights recorded.

Data analysis

Data were analyzed using a descriptive statistical package in SPSS Windows professional (version 16) and Genstat (Discovery Edition).

RESULTS AND DISCUSSIONS

Type of breeds kept, feed used, fertility determination and number of eggs laid/ bird/ year

Fifty percent of the respondents preferred and reared lavender because they are hardy in addition to meeting end-user choice. The other 50% of respondents reared the pearl because it was readily available. No respondent reared the white breed. Payne (1990) reported that there are three main breeds of guinea fowls; which includes the white, the pearl and the lavender with the pearl being the most common in northern Ghana. Table 1 shows the results of the type of feed used to feed guinea fowls. 80% of the farmers fed their birds with maize while 20% fed millet before they laid. During laying however, the reverse was true.

Table 1 - Type of feed fed to guinea fowl before and during laying

Items / Type of feed	Before laying		During laying	
	Frequency	Farmers (%)	Frequency	Farmers (%)
Maize	8	80	2	20
Millet	2	20	8	80
Total	10	100	10	100

Overall $\chi^2 = 0.000$, df = 1for before laying and the same for during laying.

In both cases the differences in frequency or percentage of farmers using one type of feed as against the other were significant (χ^2=0.000, df=1). When respondents were quizzed further as to such practice, their response was that they perceived maize as promoting growth and maintenance while millet promoted egg production. The perceptions of these farmers suggest millet ought to have a higher nutritive value than maize since laying

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requirements are higher than growing requirements. Interestingly, this perception by these farmers appears to be on a sound scientific base because work by Adeola et al. (1996) found that Indiana pearl millet had marginally higher levels of energy compared to maize (4.52kcal/g as against 4.33kcal/g) and much higher crude protein than maize (12.5% as against 8.4%).

Fertile eggs were determined using the texture of the egg shell (80%) and size of the eggs (20%). Respondents indicated that with regard to texture, shells with rough surfaces hatch well when set while those with smooth surfaces do not hatch well when set. For size of eggs, though relative, they noted that small sized eggs were not good for incubation. These perceptions by these respondents may be backed by scientific findings of Nwagu and Alawa (1995) Biwas (1999) who noted that for hatching eggs, their weights should be between 40-45g so as to give a positive relationship between egg weight and number of keets that will hatch from them. Twenty of the farmers` birds laid 50eggs, 20 % laid 80eggs, 50% laid 100 eggs and 10% laid 120 eggs per bird per year. Since 90% of the farmers' responses fell within the range of 50 -100 eggs, the findings of this study are partly in agreement with work by Dei and Karbo (2004) who reported that guinea fowl lay about 40-80 eggs in a year in the traditional system of management.

Hatchability and keets performance

Number of eggs incubated, mean weight of eggs incubated, total weight of eggs incubated, number of eggs hatched, percentage hatchability and mortality did not differ significantly (P>0.05) between the two communities (Table 2). However the Cheyohi community figures tended to be higher for the parameters considered. The similar types of management practices may be implicated in the minor variations in the results obtained.

For the backyard guinea fowl keeper, the guinea hen or more commonly the chicken hen, is allowed to sit on 10 to 15 eggs depending on her size and ability to cover the eggs effectively with her wings (Ayorinde, 1988, 1990a), however in this study the mean number of eggs incubated ranged from 18-25 (Table 2) which was much higher than what was alluded to in Ayorinde's findings above.

Table 2 - Effect of location on egg indices, hatchability and mortality

Parameters	Community		S.e.d	Sig.
	Nafaring	Cheyohi		
Mean number of eggs incubated	18.4	25.4	4.5	ns
Mean weight of eggs incubated (g)	31.4	31.8	0.5	ns
Total weight of eggs incubated (g)	577.8	807.7	140.7	ns
Number of eggs hatched	13.4	18.6	3.3	ns
Percentage hatchability (%)	72.8	73.6	6.4	ns
Mortality rate (%)	61.2	68.8	6.0	ns

S.e.d- standard error of difference, Sig. = significance, ns= non-significant.

It has been noted elsewhere in this report that the mean egg weight was lower compared to other research findings. The lower weights possibly translated into smaller sizes which could thus have made it possible for these birds to incubate larger numbers of eggs or it may well be that these birds could also have been slightly larger in size than birds used by other research or even a combination of both factors that made the incubation of the above range of eggs possible.

The mean egg weight of around 31.4–32.8g in this study fall below the recommended standards by Nwagu and Alawa (1995) and Biswas (1999) who stated that eggs for hatching should be at least weigh between 40-45g in order to obtain relative high percentage of hatchability but was close to the lower weight limits of Ayorinde's (1987c) findings that local guinea fowl egg weighs between 34 and 45g. Again Ayorinde et al. (1989) noted that egg weight in the first year usually starts at about 28g, increases to an average of 39g by the end of the first breeding season and improves slightly further in the second and third breeding seasons, hence it is also plausible that these birds could have been in the early to mid-part of their first breeding season.

The mean hatchability for this study ranged between 72.8%-73.6% which was slightly above findings by Saina et al. (2005) who reported a mean hatchability of 71% for guinea fowl eggs incubated naturally. The guinea fowl farmers in these two communities, Nafaring and Cheyohi, may thus be said to possess some reasonable experience in incubating guinea fowl eggs.

Mortality ranged between 61-69% in the two communities. Though that of the Cheyohi community tended to be higher the difference was not significant (P>0.05). A number of researchers (Mbi and Djang-Fordjour, 1998; Karbo et al., 2002) have noted that mortality is at its peaks within the first one to eight (1-8) weeks of age and this is due to poor management and health care which in turn are caused by harmful microorganism such as virus, bacterial, mould, and protozoa (Dei and Karbo, 2004). The main diseases of guinea fowls include; paralysis, gumboro, coccidiosis, pullorum diseases, worm infestation (Dei and Karbo, 2004), so management at these rural communities require substantial improvement with such high mortality figures where more than half the flock is lost at 4 weeks of age.

Mean number of keets per the 2 locations, mean daily weight gain of keets per the 2 locations and mean weekly weight gain of keets per the 2 locations were significantly different between weeks (P<0.001) (Table 3). The significant differences observed in mean number of keets from one week to the other confirms the high mortality rate that was recorded at the end of the study. Mortality in weeks one and two were highest but decreased at a

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decreasing rate for weeks three and four (Table 3) as shown by the superscripts in reduced numbers. Teye and Gyawu (2001) reported a mean daily weight gain for keets to be 5.33g in their second week which is comparable to the value obtained in this study. The same authors reported weight gain values of 6.59g and 7.70g for the third and fourth weeks which are slightly lower than the values in this study (Table 3) possibly because farmers in these communities fed the keets with only termites which are very high in protein. The significant difference ($P<0.05$) for total weight gain of keets per the 2 locations was only observed between weeks one and two, possibly because the gain in weight due to growth was higher than the rate of lost in weight due to mortality from the higher numbers of mortality recorded with time. This may have compensated for the lack of significant differences in total weight of keets between weeks two and four (Table 3).

Table 3 - Keet performance from week two to week five

Parameters	Weeks				S.e.d	Sig.
	2	3	4	5		
Mean number of keets per the 2 locations	16^{a}	11.5^{b}	7.5^{c}	5.5^{c}	1.83	***
Mean daily weight gain of keets per the 2 locations	5.2^{d}	6.8^{c}	8.2^{b}	9.7^{a}	0.33	***
Mean weekly weight gain of keets per the 2 locations	36.4^{d}	47.6^{c}	57.4^{b}	67.9^{a}	2.30	***
Total weight gain of keets per the 2 locations	579^{a}	550^{b}	431^{b}	372^{b}	90.60	*

S.e.d- standard error of difference, Sig. = significance, different superscripts ^{abcd}= in a row imply significant differences between treatments, ***= significant at $P<0.001$, *= $P<0.05$.

CONCLUSION

The pearl guinea fowl was the variety that was readily available even though lavender was preferred for rearing because it was hardy in these communities. Majority of farmers in these communities either used maize or millet depending on whether the bird was growing or laying. Texture and size of the eggs were attributes employed in assessing fertility of eggs and majority of these fowls laid between 50-100 eggs/bird/year. Also, the average egg weight in these communities was below recommended standard for hatching, leading to unacceptably high mortality rate of keets after hatching. However fertility of these eggs was not in doubt for both communities.

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DEGRADATION CHARACTERISTICS OF SOME SUDANESE GRASSES AND GAS PRODUCTION TECHNIQUES

A.O. Idris[1*], C. Kijora[2], A.M. Salih[3], I. Bushara[4], H.A.A. Elbukhary[1]

[1]*Department of Animal Production and Range, Faculty of Natural Resources and Environmental Studies, Peace University, P.O. Box 20, El Fulla, Sudan*
[2]*Department of Plant and Animal Sciences, Humboldt-University of Berlin, Philippstr.13, Building No. 9, 10115 Berlin, Germany*
[3]*Department of Nutrition, Faculty of Animal Production, University of Khartoum, P.O. Box 32, Khartoum-North, Sudan*
[4]*Department of Animal Productions, Faculty of Agricultural Sciences, Dalanj University, Sudan*

E-mail: abuelgonj2002@hotmail.com

ABSTRACT: *Eighteen plant species, three ingredients, and six diets were studied for their degradation characteristics, using gas production techniques. The palatable grasses were selected during the rainy season from the range land of Kordofan, Sudan. The ingredients were Roselle seeds, Sorghum grain and Groundnut cake. The samples were incubated for 4, 8, 12, 24, 48, 72 and 96 h, using rumen inoculum of three of the sheep used for the nylon bag. The results showed a large variation between the different plant species in the gas volume. The potential gas volume reflected the presence of anti-nutritional factors. Gas production from the ingredients indicated that sorghum grain recorded the highest gas production volume. The gas production at different time intervals showed increased degradability in the grasses, diets and the ingredients. Eragrostis tremula could be used as reference forage in evaluating the organic matter digestibility and energy density of grasses and Farsefia longisiliqua as a reference for crude protein.*

ORIGINAL ARTICLE

Key words: *In vitro, Gas production, Grasses degradability, Rangeland of Kordofan, Sudan*

INTRODUCTION

In vitro gas production from a grass sample incubated with a rumen fluid inoculums has been successfully used by Menke et al. (1979) and Steingass and Menke (1986) to predict the nutritive value of the substrate fermented. In their feed evaluation systems, the gas produced in 24 h is one parameter others are crude protein, crude fat, crude fibre and crude ash, which are used to predict metabolisable energy (ME).

There are relationships between digestibility in vivo and gas production (carbon dioxide and methane) in vitro, when the feeding stuff is incubated with rumen liquor for 24 hours. It can be used for estimation of digestibility of organic matter and metabolizable energy of grass (Steingass and Menke 1986; Manke and Steingass, 1987); Manke and Steingass, 1988. The present study was conducted with the objective of testing the degradation characteristics of some Sudanese grasses using gas production techniques to rank grasses according to their nutritive value.

MATERIAL AND METHODS

Preparation of the sample

In vitro digestibility method (the gas test, Close and Menke, 1986) was used for three ingredients, experimental diets (experiment1 and 2 rations) and 20 palatable range grasses from rangeland of Kordofan, Sudan (Tables 1 and 2). The samples were crushed to pass through 1 mm screen, and about 230 mg air dry material was placed into the bottom of a glass syringe. The feeding stuff samples were incubated in triplicates in two different days (with different batches of sheep rumen liquor) yielding 6 parallel measurements. The medium composed of micro-mineral solution, rumen buffer solution, macro-mineral solution, rezuine solution and reduction solution were prepared immediately

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before collection of rumen liquor. Rumen samples were obtained from two crossbred sheep fed on freshly cut forage and concentrate. It was collected before the morning feeding, placed in a container, sealed immediately and transported to the laboratory that needed one hour. Preparation of N rich media and rumen liquor was as described by Menke and Steingass (1988). The method used for gas production measurements was as described by Menke et al., (1979).

Rumen liquor was collected from two sheep fed on a roughage diet, homogenized, strained and filtered through glass wool to prevent N deficiency in the syringes, the liquor was mixed with the medium. Thirty ml of rumen liquor medium-mixture was pumped with automatic pipette in to each syringe. The samples were incubated in 100 ml calibrated glass syringes in two automatic incubators, basically by the procedure of Menke et al. (1979) and Steingass and Menke (1988). As a modification the syringes were incubated in an incubator (Electrically heated isothermal oven set at 39 ± 0.5°C) with holes to hold the syringes upright in it. Incubations were started in the mooring, thus the second readings were done 6 hours later. The syringes were shaken automatically during the runs. Readings were made at 4, 8, 12, 24, 48 and 72 h post-infusion. All readings were taken quickly to avoid a change in temperature. The gas production was also compared with a standard hay sample and concentrate which had been used by Steingass and Menke (1986). Calculation of gas volume results were estimated by Fit curve exile programme (Chen, 1997). The IVGP profiles were fitted to the monophasic equation of Groot et al. (1996).

The digestibility of organic matter (do %) was calculated from the gas production (Gb) and content of crude protein (XP, g/kg DM) and crude ash (XA, g/kg DM):

do =14.88 + 0.889 Gb + 0.045 XP + 0.065 XA

Similarly the content of the metabolizable energy (ME, MJ/kg DM) was calculated from the gas production (Gb) and content of crude protein (XP) and crude lipids (XL, g/kg DM) for concentrate compounds test samples:

ME =1.06 + 0.157 Gb + 0.0084 X P + 0.022 X L – 0.0081 X A

The equation for roughages test samples is:

ME = 2.20 + 0.136 Gb + 0.0057 X P + 0.00029 X L 2

Statistical analysis

The results of gas volume recordings were fitted to the exponential equation $P = a + b\,(1 - e^{-ct})$, where p is the gas volume at time t and a, b, and c are constants describing gas production with time: the constants 99 are based on gas volume recordings at 4, 8, 12, 24, 48 and 72 h. Significant differences between means with respect to gas volume readings were tested using Duncan's multiple range test (Duncan, 1955).

Table 1 - Ingredients used in the experimental rations (%) Experiment 1

Ingredients	Diet		
	A	B	C
Molasses	10	–	10
Ground nut cake	89	99	–
Roselle seeds	–	–	89
Common salt	0.75	0.75	0.75
Salt lick	0.25	0.25	0.25
Total	100	100	100

Table 2 - Ingredient used in the experimental diets (%) Experiment 2

Ingredients	Diet		
	1	2	3
Molasses	5	–	7.5
Ground nut cake	40	40	40
Roselle seeds	25	25	25
Sorghum	29	34	26.5
Common salt	0.75	0.75	0.75
Salt lick	0.25	0.25	0.25
Total	100	100	100

The results

Chemical compositions (%) of the diets were shown in table 3 and 4. The gas production at different time intervals showed increased degradability in the samples of diets and the ingredients (Table 5). In experiment1, diet A reflected the greater gas volume at different time intervals. However, diet B showed a decrease in gas volume at 4 h up to 8 h incubation, while in diet C a slight decrease was observed between 12 and 48 h incubation (Table 5). For the experiment 11 diets, diet 3 showed highest level of gas volume in intervals, 4, 8, 12 and 24 h incubation, while diet 2 was recorded lowest levels of gas volume in intervals, 4, 8, 12 and 24 h incubation, but diet1 recoded higher gas

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volume only at 48 h incubation (Table 5). Gas production from experimental ingredients was indicated that Hibiscus sabdarifa recorded lower gas production volume in the intervals, 8, 12, 24 and 48 h incubation, while grain sorghum was recorded highest gas production volume in the same intervals (Figure 1).

Gas volumes which could be attributed to rapidly fermentable fraction of the feed, a, for experiment 1 (diet A, diet B, and diet C), experiment 11 (diet 1, diet 2, and diet 3) and ingredient samples ranged from - 81.0818 to -4.9398, from - 67.6603 to 65.30115 and from -92.0139 to -5.7504 ml, respectively. Negative values pointed to the general occurrence of a lag phase in these plant species. The volume produced from the insoluble but potentially degradable, b, part of the feed ranged from 48.16 to 105.5625, 104.6856 to 111.3883 and from 48.16 to 116.25 ml for experiment 1 rations, experiment11 rations and ingredients, respectively. The potential gas volume (a + b) ranged from 24.4807 to 45.1202, 39.38445 to 43.728 and 24.2361 to 82.2783 ml for the experiment 1, experiment11 and ingredient samples, respectively.

Table 6 represents the gas production of grasses at different time intervals. The results showed increased degradability in some grasses as was reflected in the greater gas volume. However, the grass *Zornia glochidiata* showed the lowest gas volume at 4 h up to 24 h incubation, they were 3.7, 7.0, 13.2 and 21.0 ml, but *Caltropis procera* recorded higher volume at 4, 8 and 48 h .While *Caltropis procera*(flowers) and *Eragrostis termila* revealed an increase in gas at 24 h incubation. *Farsefia longisiliqua* was recorded highest gas volume at 48 h incubation (57.6 ml). The gas production of the grasses at 24 h incubation was ranged from 20.9 to 49.5 ml.

The fermentable fraction (a) for grasses ranged from −10.0468 to7.2818 ml. Also the negative values pointed to the general occurrence of a lag phase in these plant species. The volume produced from the insoluble but potentially degradable, (b), part of the feed ranged from 29.98 to 66.99 ml. The minimum and maximum potential gas volume (a + b) was ranged from21.2885 to 65.3228 ml, respectively.

Table 3 - Chemical composition (%) of the supplementations on dry matter basis (experiment 1)

Nutrient	Diet A	Diet B	Diet C
Dry matter (DM)	93.99	94.13	94.22
Crude protein (CP)	54.20	57.32	23.91
Crude fibre (CF)	5.22	5.14	16.2
Ether extract (EE)	5.05	6.18	14.47
Neutral detergent fibre (NDF)	10.53	10.71	28.48
Acid detergent fibre (ADF)	6.82	7.09	22.02
Acid detergent lignin (ADL)	1.13	1.32	7.06
Hemicelluloses HEMI	3.71	3.62	6.46
Cellulose(CELLU)	5.69	5.77	14.96
Energy density (ME, MJ/Kg DM)	13.67	12.76	9.87
In vitro OM digestibility (%)	87.74	80.78	62.17

Table 4 - Chemical composition (%) of supplementations on dry matter basis. (Experiment 2)

Nutrient	Diet 1	Diet 2	Diet 3
Dry matter (DM)	92.31	92.63	93.42
Crude protein (CP)	38.06	36.93	33.03
Crude fibre (CF)	8.95	9.79	8.77
Ether extract (EE)	5.3	6.5	8.77
Neutral detergent fibre (NDF)	19.13	19.24	20.46
Acid detergent fibre (ADF)	14.08	14.86	12.98
Acid detergent lignin (ADL)	4.51	4.43	3.95
Hemicelluloses HEMI	5.05	4.38	7.48
Cellulose (CELLU)	9.57	10.43	9.03
Energy density (ME, MJ/Kg DM)	11.25	11.29	11.71
In vitro OM digestibility (%)	73.38	71.53	71.21

DISCUSSION

Table 5 shows the gas production of the experimental diets and the ingredients. In experiment 1, diet A recorded greater gas volume at different time intervals. Diet B had lowest gas volume at 4 h incubation time, while diet C recorded lowest gas volume at 12, 24 and 48 h incubation. It was observed that diet A was superior from the other diets, diet A had high percentage of groundnut cake which represent source of energy (Table 1). Also diet A had highest in vitro OM digestibility followed by diet B and C. This may be due to the crude protein content which increased in diet A and then increased the digestibly (Table 3). These results were in line with the findings of Bahatta et al. (2002) and Guimaraes-Beelen et al. (2006), who reported that, the increase in gas production may be attributable to both protein and carbohydrate fermentation.

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Table 5 - Gas production (mean ±S.E) ml per 200 mg dry sample from incubation at different time intervals

Diets and ingredients	Incubation time (h)					Constants			
	4	8	12	24	48	a	b	c	a+b
Diet A	23.8±1.13	37.1±1.17	43.1±1.10	48.5±0.98	51.3±1.51	–4.9398	50.0600	0.1093	45.1202
Diet B	16.4±1.20	29.1±1.15	35.3±1.23	39.3±0.99	43.8±1.41	–5.7504	48.16	0.1565	42.4096
Diet C	22.2±1.10	29.4±1.19	30.9±1.20	30.5±1.00	37.6±1.34	–81.0818	105.5625	0.5611	24.4807
Diet 1	18.5±0.78	30.4±0.99	37.1±1.10	41.0±0.99	44.4±0.99	–66.1004	107.2198	0.4603	41.1194
Diet 2	15.5±0.98	28.2±0.65	36.0±0.64	39.9±0.65	43.0±1.02	–67.6603	111.3883	0.4812	43.728
Diet 3	20.1±0.34	31.4±0.97	37.7±1.23	41.5±0.89	42.2±1.14	65.30115	104.6856	0.49664	39.38445

a, b and c represent constants in the equation $P = a + b(1 - e^{-ct})$ describing gas production with time: the constants are based on gas volume recordings at 4 ,8 ,12 ,24 , 48 and 72 h)

Table 6 - Gas Production (mean ± S.E) (ml) from incubation of different types grasses at different time intervals

Grasses species	Incubation time (h)					Constans			
	4	8	12	24	48	a	b	c	a+b
Accaacia monifera	12.7±2.21	23.8±2.91	32.8±3.56	40.1±3.55	45.6±3.59	-4.9398	50.06	0.1093	45.1202
Eragrostis tremula	21.0±1.40	36.1±1.31	42.9±0.38	49.5±1.24	53.0±1.28	-4.8117	56.93	0.1534	52.1183
Farsefia longisiliqua	10.3±0.80	17.8±1.04	26.5±1.82	43.7±1.75	57.6±2.18	-1.6672	66.99	0.0457	65.3228
Chascanum marrubifolum	14.1±0.94	25.2±1.36	36.2±0.94	44.7±1.21	50.8±1.97	-4.8606	55.47	0.1031	50.6094
Euphorbia aegyptiaca	14.9±0.27	25.1±0.27	32.6±0.66	38.6±0.98	40.6±1.74	-3.9911	44.57	0.1366	40.5789
Ipomoea cordofana	17.0±1.49	27.3±3.68	31.9±4.56	42.5±2.64	51.2±1.81	7.2818	45.47	0.0657	52.7518
Echinochloa colonum	4.9±0.85	13.0±0.88	21.5±0.89	37.8±0.64	46.7±1.59	-8.5713	58.95	0.0607	50.3787
Zornia glochidiata	3.7±0.60	7.0±1.63	13.2±1.61	21.0±0.88	24.6±2.16	-4.4773	30.53	0.0695	26.0527
Trebulus terrestris	11.0±0.33	21.6±0.69	29.2±1.06	36.1±0.92	40.0±1.59	-6.2986	46.01	0.1181	39.7114
Gisekia Pharnacoides	12.0±0.14	21.2±0.49	25.0±0.70	28.6±0.90	31.2±3.33	-3.3325	33.74	0.1544	30.4075
Polygala erioptera	18.0±0.45	30.4±0.64	38.7±0.94	47.3±1.25	52.1±1.80	-1.0154	52.78	0.1129	51.7646
Abadaib spp	18.5±0.76	31.7±0.91	35.9±0.50	43.0±0.13	47.7±0.73	1.2589	45.45	0.1250	46.7089
Indigofera spp	8.8±1.151	19.0±1.90	25.1±1.97	32.1±2.70	32.3±3.90	-10.0468	42.85	0.1437	32.8032
Sesamum alatum	8.2±0.86	14.6±1.03	19.7±1.28	20.9±2.51	21.1±3.19	-8.6915	29.98	0.2037	21.2885
Zaleya pentandra	17.1±0.81	30.2±0.88	36.7±0.53	41.3±0.54	44.8±1.01	-6.8842	50.68	0.1618	43.7958
Belpharis ciliaris	13.9±0.42	24.7±0.44	32.1±0.33	40.1±0.52	45.0±0.91	-2.0652	46.89	0.1053	44.8248
Amaranthus viridis	19.3±1.51	34.0±1.51	41.0±1.68	46.4±1.98	51.3±1.96	-5.5519	55.41	0.1511	49.8581
Caltropis procera(flowers)	26.1±1.05	41.4±0.91	45.9±0.96	49.2±0.83	53.3±0.64	-5.5316	57.02	0.2046	51.4884

a,b and c represent constants in equation $P = a+b(1-e^{-ct})$ describing gas production with time: the constants are based on gas volume recordings at 4 ,8 ,12 ,24 , 48 and 72 h).

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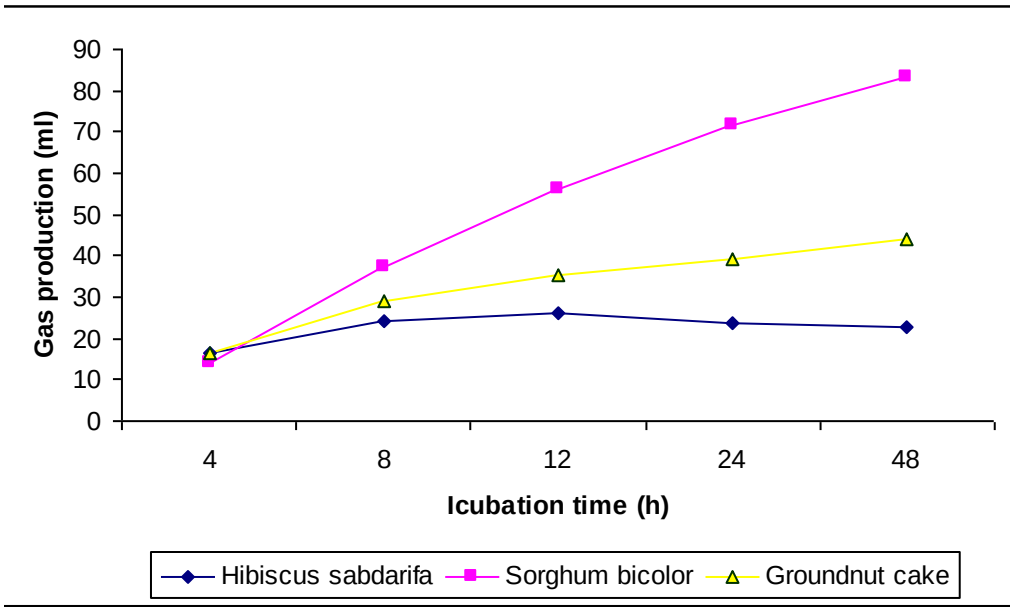

Figure 1 - Gas production from experimental ingredients

Diet C was composed of 89% of Roselle seeds (*Hibiscus sabdarifa*), pure Roselle seeds recorded lowest gas volume, when compared with the other ingredients. The lowest volume of the gas in diet C due to Roselle seeds that, contain lower crude protein (Table 3). The chemical composition of diet C showed that, it has highest Hemicelluloses and Cellulose content than the other diets (Table 3). Both Hemicelluloses and Cellulose represent anti-nutritional factors, this result is similar to that reported by Ahmed and El-Hag (2004) and Khazaal et al., (1993), who recorded that, anti-nutritional factor decreased the digestibly of the dry matter.

High hemicelluloses and cellulose content of Roselle seeds might explain the lower organic mater digestibility observed in diet C, through a decrease in rumen microbial activity .This explanation is in line with findings of Carvalho et al. (2005). This explanation is not acceptable for groundnut cake. In this case, the response observed could be eventually attributed to its lower gas volume in late incubation time (Figure 1) additionally; the relatively high content of cell wall structures (hemicelluloses and cellulose) might restrict microorganism activity, and then lowered gas volume.

The estimated metabolizable energy (ME) content of the supplements reflects their gas production level, crude protein and crude lipids content. Diet A and diet C, have the highest and the lowest energy content, respectively. It was observed that, diet A recorded highest gas at24 h, followed by diet B and diet C which had lowest gas level. Energy density is affected mainly by gas level. In experiment II, the results show that energy densities in the three diets were similar, because the differences in gas volume at 24 h were 41, 39.5 and 41.5 ml for diet 1, diet2 and diet 3, respectively (Table 5).

Diet 3 showed highest gas volume in 4, 8, 12 and24 h incubation, while diet 2 recorded lowest gas volumes in the same incubation intervals, but diet1 recoded higher gas volume only at 48 h incubation. Diet 3 showed the best gas volume, because it has higher molasses than diet 1 (Table 2). The gas production at 8, 12, 24 and 48 h showed highest degradability in grain sorghum that was reflected in the greater gas volume. While *Hibiscus sabdarifa* showed lowest gas production amongst the ingredients, which could be due to the presence of certain anti-nutritional factors (Figure 1). Sorghum is rich in structural carbohydrates that contain high energy (Molina Alcaide et al., 2003), so it produced more gas than other ingredients (protein sources).

The gas production of grasses at different time intervals is shown in Table 6. The results showed increased degradability in some grasses as was reflected in the greater gas volume. However, *Zornia glochidiata* showed the lowest gas volume at 4 h up to 24 h incubation, this explains that *Zornia glochidiata* had lowest energy content. *Eragrostis termila* revealed an increase in gas at 24 h incubation, so it recorded the highest energy. *Farsefia longisiliqua* recorded highest gas volume at 48 h incubation. This result may be due to the maximum potential gas volume (a + b). It may also be explained by a high solubility, or degradability of *Farsefia longisiliqua*. Other studies have shown similar explanation Ahmed and El-Hag, (2004). *Sesamum alatum* had lowest gas volume at24 h. This is due to minimum potential gas of the grass. In fact, the gas level at 24 h had direct effect to energy content of the grasses (Table 5). Some samples recorded higher potential gas volume they were reflecting the presence of less anti-nutritional factors. These findings are in general agreement with Ahmed and El-Hag (2004) and Bahatta et al. (2002) , they found that, the potential gas volume (a + b) was higher for the samples treated with polyethylene glycol, reflecting the presence of anti-nutritional factors. These were higher for legumes than tree pods or grasses.

In this study, the gas production of grasses at different time intervals recorded higher levels compared with the study carried by Ahmed and El-Hag (2004). The variation between the two studies may be due to many factors, such as grasses species, varieties, season and rain fed, all these factors may affect the plant components. The gas

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production results of grasses are in agreement with Kubuga and Darko, 1993) and Blümmel and Ørskov (1993).They used both the nylon bag and in vitro techniques and found valuable assistance in evaluating the quality of a large number of forage samples.

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INVENTORY AND DEVELOPMENT PERSPECTIVE OF MILK PRODUCTION IN SAHARAN AREA: THE CASE OF THE GHARDAÏA REGION (ALGERIA)

H. BENSAHA[1], R MAYOUF[2]*, L BENSAHA[1]

[1]*Applied Research Unit in Renewable Energy, Ghardaia 47000 Algeria*
[2]*Institute of Agricultural Sciences, Hadj Lakhdar University, Batana 05000, Algeria*

*Email: rabahmayouf@gmail.com

ABSTRACT: *The National Fund for the Development of Agricultural Investments (FNDIA) supports various actions, including the dairy industry (mini-dairy, production and birth bonuses, milk collection, processing and artificial insemination). At the level of the Ghardaïa region, like the other Saharan regions, FNDIA helped initiate the development of livestock and thereby contributed to the increase in the number of head of cattle. The establishments of nurseries and of specialized dairy barns have created a dynamic in the dairy cattle farming and have positive impacts on the local market, namely an increase in the production of milk. According to the Directorate of Agricultural Services (DSA) of the Wilaya of Ghardaia (2010), the number of imported dairy cattle between 1995 and 2010 rose from 177 to 1688 dairy cows owned by the private sector. 13 400 liters of milk are collected daily by dairies and milk collection points. In this context, the objective of this research is to develop an inventory of the dairy industry in Ghardaia and identify its strengths and weaknesses in order to propose solutions to ensure its sustainability and thus provide guidance to the strong investment by government.*

ORIGINAL ARTICLE

Key words: *Agricultural Development, Dairy Cattle, Ghardaïa, Milk Production, Saharan Region*

INTRODUCTION

Development of milk production is among the priorities of the Algerian state, to meet a growing demand for milk and its derivatives and, in particular, to cover the deficit in animal protein, facing a spiraling population growth. The overall need for milk of Algeria in 2007 was estimated at 5 billion liters, with an average consumption of about 130 liters per capita per year (I.T.E.L.V. 2007).

Various national programs have been initiated since the 90s and are to encourage and induce a series of policies to upgrade the local milk production, to promote self sufficiency (Mamine et al., 2010). This one will improve the protein intake of the local populations concerned (Bensaha, 2008) and will develop an economic sector that can be prominent in the development of the Saharan areas. The development of this sector creates jobs and wealth (Ouakli et al., 2003).

All state aid and interventions planned under the plan slag quickly created a craze for cattle including dairy cattle became part of the socio-economic landscape of the Saharan territories. These measures have largely contributed to the establishment of stable performance in these areas.

Of the 1.56 million which heads up the national herd, found traditionally in the regions north of the country about 80% of the cattle scattered irregularly, with 59% in the east, west 14% and center 22%. Only 5% are located in the Saharan regions, the equivalent of 77 000 head of cattle (Senoussi et al., 2010).

In this context two questions arise:

- What is the current position of dairy farming in the region of Ghardaia?
- What are the dynamics into play?

This study aims to answer these key questions, addressing aspects of production, livestock management, packaging, distribution, consumption and marketing, to begin thinking about the potential of improvement of this sector in this region.

Crop production and the feed balance current

In Algeria, the land involved in forage production is nearly 40 million hectares (Mammeri, 2003). They represent barely 7% of the useful agricultural area (SAU). One of the most striking consequences of this lack of

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fodder SAU, is the weakness of milk production. Indeed, it depends, in large part, forage production (Abdelguerfi et al., 2003). It seems obvious at this stage, taking into account the importance of the actual availability of food resources in the projection of development activities (Sraïri 2004).

Therefore, the state has established grants to encourage the farmer to produce himself necessary food for his animals, such as forage and grain. These grants were provided for the purchase of irrigation equipment and bonuses 5000 DA / ha have been granted for the installation of high forage yields and feed value optimal. Emphasis was placed on the development of forage seed production quality, adaptable to our climate, focusing on ways to produce ensilage and build silos.

In the region of Ghardaia, the total agricultural area only 16% of the total area of the Prefecture, due to the dominance of unfavorable geomorphological. Thus, 84% of the area consists mostly of unusable surfaces, they include not only unproductive land not used for agriculture, but also areas that cannot be grown or processed in background and, more definitive , surfaces covered by urban areas, various buildings and communications channels (Bensaha 2008). This is combined with factors such as drought, water shortages, inadequate control of operating techniques, the high cost of cattle feed and fodder lack perimeters, which are factors that limit the development of dairy farming.

This stems from that culture and forage production in Ghardaïa remains, in many respects, a marginal farms. Indeed, the proportion of land reserved for forage crops, used extensively moreover, remains low. (Table 1)

Table 1 - Main crops harvested and implanted in the wilaya of Ghardaia (DSA Ghardaïa. 2010)

Cultures	Areas carried out (ha)	Quantities harvested (qx)	Average yield (qx / ha)
Cereals	1 150	47 384	41,20
Industrial Crops	476	9 520	20
Fodder	1 900	366 700	193
Market Gardening	2 666	400 000	150
Potato	130	26 800	206,15
Arboriculture	3 237	117 600	36,33

The incentive for the production of fodder by the breeder, rehabilitation and diversification of the forage crops, especially those consumed fresh, through a careful selection of forage species adapted to local conditions, the use of 'adequate fertilization to improve forage production in quantity and quality, are needed (Mayouf 2008).

Workforce data Dairy

Cattle breeding have an important economic and social role in Algerian society. Indeed, the dairy sector is strategic in view of its impact on food security and its place in the socio-economic. Furthermore this importance, the dairy sector supports the maintenance of livestock on their farms by providing a regular income. It thus contributes to the intensification and integration of agriculture in national economy.

This speculation has risen in 20 years, from a casual family breeding to an interest preeding, by the orientation and awareness of supported dairy producers by grading the health of their business and their introduction into the sector « milk ». The activity of dairy cattle in the region of Ghardaia plays, in fact, a much larger role that cannot lead one to believe the simple statistical reading of the part that plays in the overall development of the region. We notice that the number of breeders is continually evolving, and this is due, no doubt, supports and subsidies from the state to place different types of farms including dairy cattle (Figure 1).

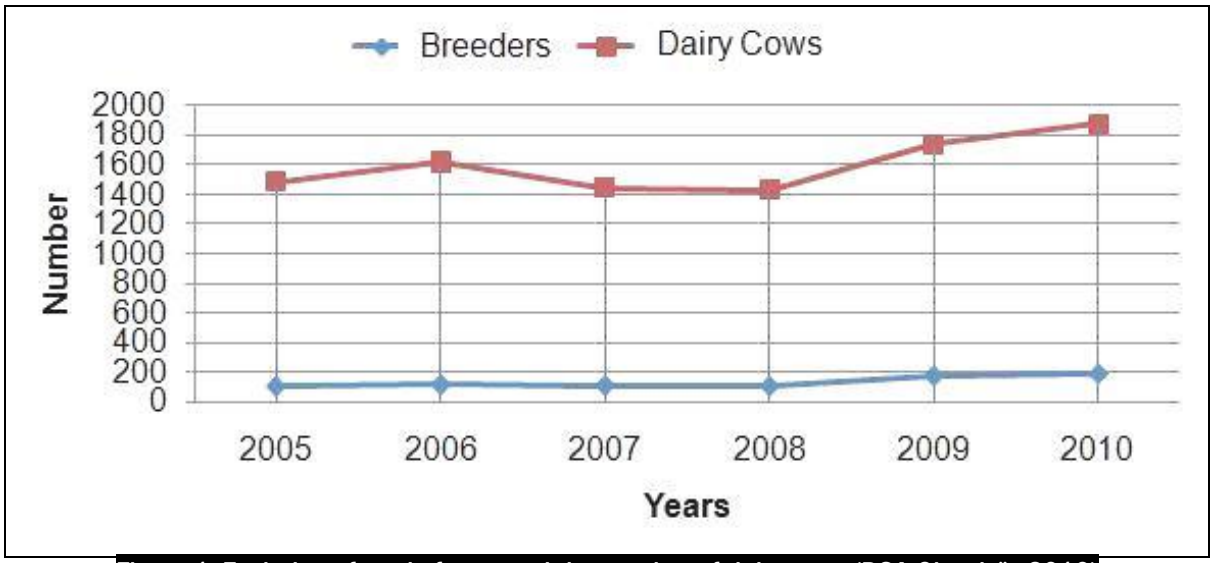

Figure 1. Evolution of cattle farms and the number of dairy cows (DSA Ghardaïa 2010)

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In the Wilaya of Ghardaia, livestock-oriented dairy is based mainly on cattle and goat. Indeed, it was counted a total number of dairy cows of about 1,688 heads, for a number of dairy goats, which is 132 heads. About 80% of farmers are moving towards cattle and 20% associate the farm to that of goats (Ouled hadj youcef et al., 2007), while ensuring strict compliance with health standards (particularly regarding the prevention of brucellosis). We should know that the rise in recent years is the direct result of increased enrollment by importing heifers, the strict application of preventive health plans, and the gradual improvement of production techniques. It is it is in this perspective that the National Agricultural Development Plan (NADP) initiated in 2000 through the dairy cattle section, has impacted positively on the Saharan space (Senoussi et al., 2010).

This type of farming livestock intensively conducted exclusively for milk production. The main livestock bred are those imported such as Holstein, the Montbeliarde the Flekvy and Brune des Alpes. The latter require a good command of livestock (buildings, balanced diet and health monitoring, ...). With a reasoned and rational conduct, dairy cattle, on both numbers of staff, is an asset capable of generating a dairy capable of ensuring self-sufficiency in consumption

Availability of labor

The need for labor is important to the cattle breeders, goats, camels or institutions related to the sector (Table 2). In the availability of the workforce, we record that over half of cattle farms face difficulties in its timely availability, because it consists of a young workforce, with no experience and with low qualifications, young non-degree holders training orienting default to livestock.

Table 2 - Labor generated in the dairy industry (DSA Ghardaïa 2010)

Dairies	71
Collectors	12
Cattle breeders	221
Goat breeders	35
Camel herders	06
Workers in institutions breeding	371
Total	716

Ability of milk collection

The wilaya of Ghardaia is a large pool that has a dairy herd assessed in 2009 to 2,630 head of cattle including dairy cows 1560 against 1320 in 2008. The assessment of management of agricultural services (DSA) reported a production of 18 million liters of milk during 2009 against 17.3 million liters in 2008. This growth is related to the advent of the policy of agricultural and rural renewal in 2008, which led the agriculture sector to focus on strengthening local production, including those of wide consumption.

Within this framework, a process incentive for all stakeholders in the sector is being implemented, including grants and aid programs consistent. This new strategy was soon proved to be successful. Thus, production levels in raw milk only increases year by year. The products of the milk collection grew significantly thanks to state support, which increased from 7 dinars to 12 dinars per liter. In 2009, the collection capacity has exceeded the 13,400 liters / day.

o bring this production to the industrial units, a small network of collectors has been created and approved the health plan. They collect milk on farms with small vehicles and refrigerated transport unit level packaging. These are solicited based on their packaging capabilities.

The increasing number of collectors and the quantities of milk machined displays a remarkable parallelism which confirms that the efforts in the formal collection were the major component of the dynamics of supply to industrial units (Figure 2).

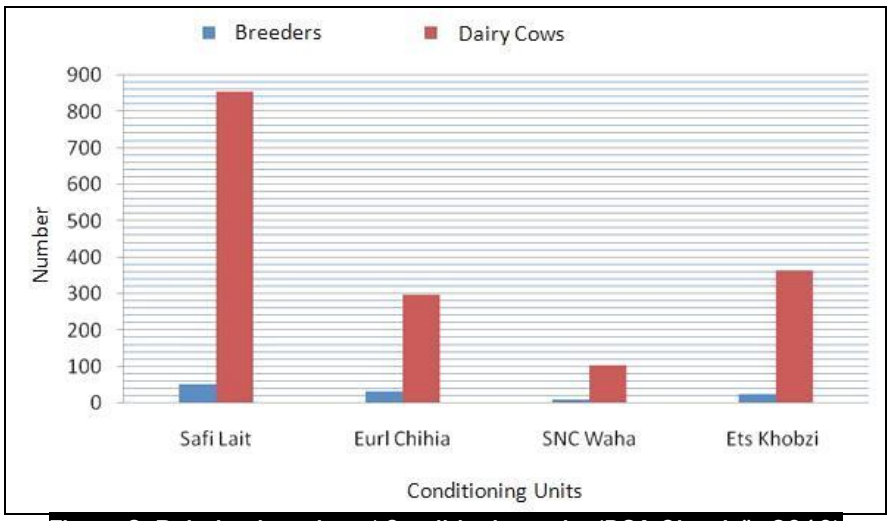

Figure 2. Relation breeders / Conditioning units (DSA Ghardaïa 2010)

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The goal of regulators was to allow these farms located throughout the wilaya of their daily flow perishable product to the packing units. But the needs remain great, and efforts must continue to be absolutely oriented collection, particularly in terms of reducing mobilization costs of raw milk in the industrial process, because, currently, processors admit that the cost of raw milk is unusually high, by between 40 and 45 DA / liter, which is why they resort to import milk powder.

It should be noted that the collection of milk is only a sideline and the breeder cannot count on to live with his family's income it generates.

Marketing and dairy industry

The nutritional status of a population is closely linked with the quality of its diet (Araba et al., 2001). The objectives of the plan in terms of milk consumption could be considered "excessively high" since they are 130 liters per capita per year, against 70 liters per capita per year in the Netherlands. They could not be reached, because consumption levels are closely linked to socio-economic household as well as culinary traditions.

The major objective through different types of milk marketing channels is to regulate the milk market to meet this issue, and ensure the smooth operation between the various links in the chain (Diagram 1):

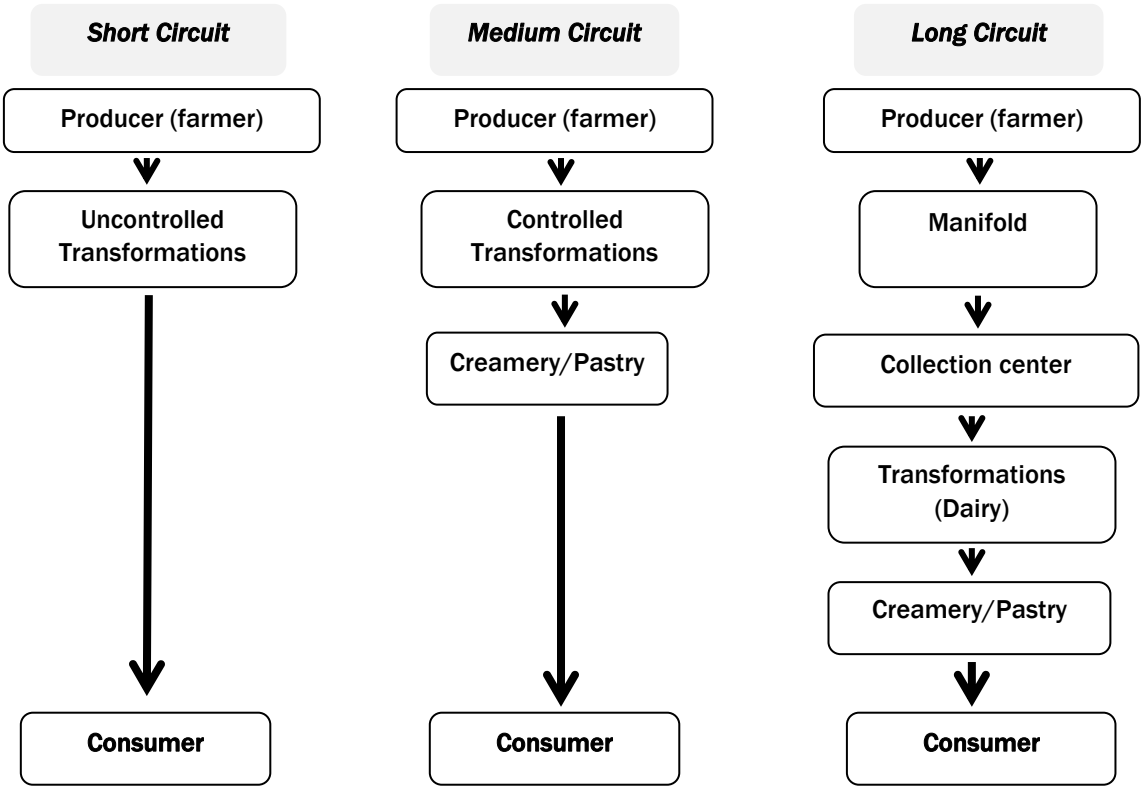

Diagram 1. Main marketing channels for milk in the region of Ghardaia. (Ouled youcef and al 2007)

The distribution of milk in bags, including through the mobilization of refrigerated transport and approved the health point of view, is gradually improving in boosting the regular circuit compared to the informal to the great satisfaction of households. In fact, it is much more quality than quantity, which now seems compelling (Sraïri, 2010).

In the region of Ghardaia, most consumers appreciate fresh products (fresh milk, Lben, kémaria ...). Among the strengths of the dairy industry include local expertise inherited over many generations of cheese-making tradition (Kémaria). This tradition has grown over the years by advances in technology, but consumers remain committed to the regional product.During the holy month of Ramadan, there is an increase in consumption resulting in higher demand from dairies. This situation causes the depletion of stocks of dairies in the wilaya. Also, the supply of milk powder should be strengthenedHealth monitoring of herds

The State, in order to guarantee public health entrusted inspection dairy veterinary services located in wilayas. These services have continued to implement measures to monitor the health of dairy cattle and especially in the fight against tuberculosis and brucellosis, two diseases that also threaten consumer health (Benkirane 2001).

It should be noted the positive impact of health action, which takes place every six months and is associated with periodic epidemiological investigations to maintain all health indices at thresholds satisfactory.

A screening program was implemented by the veterinary inspection of the wilaya of Ghardaia to limit the rise of zoonotic diseases mainly due to the consumption of milk that could escape the circuit imperative pasteurization unit level packaging.

Development institutions concerned have strengthened supervision and support for farmers by 40% of veterinary officials, 30% of private veterinarians and 12% in pre-employment (Table 3), in order to undertake

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extension activities in an area where, precisely, professionalism is required because of the complexity of the activity (Amellal 2000)

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Table 3 - Veterinary medical coverage of wilaya (DSA Ghardaïa 2010)	
Officials veterinarians	16
Pre-employment contract	05
Para-veterinary officers	07
Private veterinary medical	12

Milk Sector Weaknesses

The dairy industry in the region of Ghardaia, reveals a number of advantages but also shortcomings surmountable. Among the constraints, it turns out that some breeders do not take into enough account of the quality of food and resort to a rudimentary form of rationing. They use as main pastoral resources, including drinn (*Stipagrostis pungens*), the Diss (*Imperata cylindrica*), the Agga (*Zygophyllum album*) and quackgrass (*Synapsis arvensis*). This, equally, without considering the nutritional value of these respective foods.

In fact, breeder are almost always forced to use dry food, because of the lack of land used for forage crops, on the one hand, and lack of irrigation water on the other. But the high cost of this type of food, bran, VL15, maize, and the scarcity of green fodder in winter, leading farmers to use more highly concentrated food, including date rubbish, bran and VL15, (representing almost 74% of cases considered during the investigation). Indeed, the forage system based on dry forages (hay and straw), and concentrated food, lead cow fattening and, therefore, a significant drop in milk production.

Forage production in the region of Ghardaia, is very low compared to the needs of livestock, which requires appropriate actions for the development of the dairy herd. Food that is the most important parameter in operating costs of milk production is also one of the most effective tools for controlling this production, both in quantitative, qualitative and economic. Thus, the food forage remains the main limiting factor, nearly 97% of the farms studied are not self-sufficient in fodder.

Regarding the actual Dairy and its adaptation to the Sahara, we find that climatic factors generally act negatively on the performance of imported breeds of cattle, which was already known. Cattle performance decrease, since much of their metabolism provides the energy consumed by the need to adapt to environmental factors (Nedjraoui, 2003). Breeds recently imported, introduced to improve production, are faced with ecological conditions quite different to those of their country of origin. Indeed, the Saharan climate is unfavorable for these animals, and deprives them of abundant food because of the lack of grazing suburban. Summer heat that exceeds the average of 34 ° C also affects milk production, because over the thermal interval [27 ° C - 30 ° C], animal productivity drops significantly (Senoussi, 2008).

As for the ability of milk collection, the main link between production and the dairy industry, it exceeded 13,400 liters / day in 2009. This volume, a marked increase, is a response to the encouragement of the State, but the collection capacity has not been able to progress from one way and it undergoes significant and important annual variations. It is estimated, therefore, that the major problem of production of milk by producers in the wilaya of Ghardaia, lies in the inadequacy of the collection of raw milk.

In terms of sanitation, modern dairy cows are both sensitive and demanding. Susceptibility to certain diseases, and requirements vis-à-vis farming conditions, maintenance of the animal and livestock buildings. Indeed, in the absence of an adequate plan prophylactic measures and hygienic routine, we found, in most farms, cases of abortions during the sixth or seventh month of gestation.

In terms of health monitoring, it is necessary to predict biannual and annual screening against major zoonotic bovine. And not forgetting the various vaccinations, for cattle, sheep and goats. The various pathologies induce loss of production and marketing of milk because of the legal prohibition of delivery, straight to drug treatment, and the farmer bears additional expenses entailed by the reinstatement of dairy cows.

Finally, the difficulty of recruiting the necessary labor, wage conditions because of discouraging and lack of interest of people for careers in farming, are among the factors hindering the development of the dairy industry in the region. Finally, the industrial production capacity of milk and dairy products have seen notable expansion in the region of Ghardaia, but the dairy industry is still not able to respond adequately to rising demand.

Recommendations and proposals

Development institutions shall, while implementing a strategy urgent deployment (scale wilaya), continue their efforts in various fields, such as genetic improvement and control of cattle feed to objective to make proposals to the government in charge of livestock, ranchers and development workers.

From this perspective, the ITELV the CNIAAG, the ONDEEC, might be called upon to construct, together with veterinary services at the wilaya, a strategic approach to sustainable development challenges at the various links the sector and ensure its sustainable development. For this, the state must invest in the crenel "Recruitment of veterinarians and animal scientists, engineers' real capital so called the cornerstone of development policy.

Also, better knowledge about the composition of herds, the comparative performance of different races in the Sahara and on forage production, are also needed. This is possible only by the experimental trials involving research centers and universities specializing in this area to: improve feeding by balanced basal rations (legumes and grasses), to optimize yields and methods of conservation and distribution of different forage species acclimated.

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CONCLUSION

In our country where the promotion of specialized dairy farming is relatively new, the Animal Research adapted to dairy farming Sahara still have several challenges. Quantity of raw milk, Dairy farming could not keep up with demand: it is still relatively low yield, probably due to the lack of logic of intensification and integration of this local production. Species of sheep and camel, thanks to their hardiness and their good adaptation to environmental conditions, may represent a crenel research to significantly improve milk production. A debate on the model of dairy farming in Algeria in the Sahara, and the place to be taken by the selection of local breeds in such environments would be an important contribution.

The definition of the different pathways that the dairy industry can guard against any dogmatism and simplification. Every decision at each level and each situation must result from arbitration must take into account all the technical and socio-economic actors.

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RESIDUE DEPLETION OF SULPHADIAZINE AND TRIMETHOPRIM IN PIGS AND BROILERS AFTER ORAL ADMINISTRATION

P. RONCADA[1]*, L. TOMASI[2], F. SORI[1], A. ZAGHINI[1], A. ZACCARONI[1], D. FERRARA[1]

[1]Department of Veterinary Medical Sciences, Faculty of Veterinary Medicine, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia, Italy
[2]Cardiovascular Department, Sant'Orsola-Malpighi Hospital, University of Bologna, Via Massarenti 9, 40010 Bologna, Italy

* E-mail: paola.roncada@unibo.it

ORIGINAL ARTICLE

ABSTRACT: *The residual behaviour of a sulphadiazine (SDZ) and trimethoprim (TMP) combination was studied in fourteen pigs and twenty-eight broilers. The drug combination was added in the amount of 700 mg kg^{-1} (SDZ) and 140 mg kg^{-1} (TMP) to pig and 300 mg kg^{-1} (SDZ) and 60 mg kg^{-1} (TMP) to broiler feed, respectively. The medicated feeds were supplied for 5 consecutive days. The tissue SDZ/TMP concentrations were measured by a HPLC method. To ensure safe residue levels in all target tissues, withdrawal time of 8.6 days and 6.0 days should be applied to pigs and broilers, respectively, treated with SDZ and TMP in feed.*

Key words: *Sulphadiazine; Trimethoprim; Pigs; Broilers; Residues; Withdrawal Time; Veterinary Drugs*

INTRODUCTION

Combinations of sulphadiazine (SDZ) and trimethoprim (TMP) are commonly used for the treatment of respiratory, gastrointestinal and urogenital infections in food producing animals. The large scale of application of this combination has led to the occasional occurrence of residues in edible tissues. These residue values could be particularly high presenting a hazard to human health if the recommended withdrawal times are not respected. The European Union allocated the two molecules in Table 1 of the Commission Regulation of the European Union (EU 2010). The MRLs fixed for pig and broiler tissues are 100 µg kg^{-1} and 50 µg kg^{-1} for SDZ and TMP, respectively.

For these two drugs, alone or in combination, several pharmacokinetic studies have been performed in pigs after intravenous (Nielsen and Rasmussen, 1975; Luther, 1979; Friis et al., 1984a,b; Gyrd-Hansen et al., 1984; Nouws et al., 1989) or oral administration (Søli et al., 1990; Nielsen and Gyrd-Hansen, 1994; Garwacki et al., 1996). The SDZ/TMP combination pharmacokinetic behaviour was also described after intramuscular injection or transdermal delivery (Sekido et al., 1992). Nevertheless, it is difficult to find information about the tissue distribution and tissue residue depletion of both compounds in this species. Similarly, notwithstanding their intensive use, very few published data are available on the pharmacokinetics and residual behaviour of these drugs in poultry (Loscher et al., 1990, Takahashi et al., 1991, Dagorn et al., 1992).

The aim of this study was to evaluate the residues of SDZ and TMP in pig and broiler edible tissues after oral administration of the two-drug combination in the feed under practical conditions. Based on the tissue residues, the withdrawal time of the combined drugs was calculated according to Guidelines of the Committee for Veterinary Medical Products of the European Agency for the Evaluation of Medicinal Products (EMEA, 1995).

MATERIALS AND METHODS

Animal treatment

Pigs: fourteen 60-day old pigs (Large White x Landrace) weighing 19.3±1 kg (mean body weight±SD) were obtained from a local farm. The animals were randomly allotted to 3 experimental groups (four pigs/group). Two pigs were used as controls. The pigs were housed in single boxes under controlled temperature and humidity. The animals were permitted *ad libitum* access to feed and water throughout the experiment. The feed and water consumption of each animal was determined every day. After 15 days of acclimatisation, SDZ and TMP were administered to pigs in the three experimental groups at 700 mg kg^{-1} and at 140 mg kg^{-1} of the diet, respectively. The medicated feed was supplied for 5 consecutive days. The time of slaughter was fixed at 1, 7 and 10 days after the end of the treatment. At each time point one group of animals was sacrificed and samples of the target tissues

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(muscle, liver, kidney and skin/fat) were taken and stored at –20 °C. At the last time point, also the control pigs were slaughtered.

Broilers: twenty-eight 45-day old broilers (Golden Comet) weighing 0.52±0.02 kg (mean body weight±SD) were obtained from a local farm. The animals were randomly allotted to 4 experimental groups (six broilers/group). Four broilers were used as controls. The broilers were caged individually under controlled temperature and humidity. The broilers were permitted *ad libitum* access to feed and water throughout the experiment. The feed and water consumption of each animal was determined every day. After 15 days of acclimatisation, SDZ and TMP were administered to the broilers in the four experimental groups at 300 mg kg^{-1} and at 60 mg kg^{-1} of the diet, respectively. The medicated feed was supplied for 5 consecutive days. The time of slaughter was predetermined at the 1, 3, 5 and 10 days after the end of the treatment. At each time point one group of animals was sacrificed and samples of target tissues (muscle, liver, kidney and skin/fat) were taken and stored at –20 °C. At the last time point, also the control broilers were slaughtered.

The study was carried out in observance of legislation concerning the use of animals for experimental purposes (D.L. 27/01/1992 no. 116).

Analytical procedures

Reagents: Sulphadiazine sodium salt and trimethoprim base were obtained from Sigma-Aldrich (Milan, Italy) and used to prepare the reference standard solutions. Methanol, acetonitrile and water, purchased from Mallinkrodt Baker (Deventer, the Netherlands), were of HPLC grade. Sodium chloride (NaCl), potassium phosphate (KH_2PO_4), sodium acetate (CH_3COONa), 85% orthophosphoric acid (H_3PO_4), 37% hydrochloric acid (HCl), purchased from Analyticals Carlo Erba (Milan, Italy), were of analytical grade. Clean-up cartridges (SPE-C18, 500 mg, 7020-06) were from J.T. Baker (Phillipsburgh, N.J. USA).

Solutions: a 0.02 M KH_2PO_4-buffer solution (pH 3) was prepared by dissolving KH_2PO_4 (2.72 g) in water (1 L); the pH was adjusted with H_3PO_4 (85%). A 0.025 M KH_2PO_4-buffer solution (pH 4.5) was prepared by dissolving KH_2PO_4 (3.40 g) in water (1 L); 500 mL of this buffer solution was adjusted to pH 3.5 by adding concentrated (85% w/v) H_3PO_4. 0.1 M HCl was prepared by diluting 0.83 mL of concentrated (37% w/v) HCl with 100 mL of H_2O. 0.5 M NaCl was prepared by dissolving 29.11 g L^{-1} and adjusting pH to 2.5 with 0.1 M HCl. 0.2 M CH_3COONa was prepared by dissolving 16.41 g L^{-1} in water. The HPLC mobile phase was made by mixing 0.02 M KH_2PO_4-buffer solution (pH 3) and CH_3CN at the ratio of 80:20 (v:v) and 83:17 (v:v) for SDZ and TMP analysis, respectively.

Standard solutions: stock standard solutions of the two drugs (200 µg mL^{-1}) were prepared separately by dissolving 10 mg of SDZ with 50 mL of methanol and 10 mg of TMP with 10 mL of methanol subsequently diluted to 50 mL with water. Both stock standard solutions were stored at –20±1 °C; under these conditions their stability is 1 month. The working standard solutions were made by diluting aliquots of the stock solutions in a 0.02 M KH_2PO_4-buffer (pH 3) to obtain concentrations ranging from 0.05 to 2 µg mL^{-1} for SDZ and from 0.02 to 1 µg mL^{-1} for TMP. Fortification solutions, containing SDZ at 0.5, 1, 5, 10 or 20 µg mL^{-1} and TMP at 0.2, 0.5, 1, 5 or 10 µg mL^{-1}, were prepared in water from stock solutions. Fortification was carried out by adding 50 µL (for SDZ) and 40 µL (for TMP) of these solutions to 1 g of the homogenised tissues.

Sample preparation and clean up: target tissues were cut with scissors to obtain small pieces and 1±0.1 g of tissue was weighted. To this amount of sample, was added water (1 mL) and methanol (3 mL), and was homogenised with an Ultraturrax (IKA Labortechink). The following steps differed for the SDZ and TMP assay.

Sulphadiazine: the homogenate was centrifuged (15 min) at 1000 x ***g*** (Beckman GPK). The supernatant was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 20 mL of 0.5 M NaCl (pH 2.5) and applied to a SPE-C18 cartridge prewashed with methanol (2 mL), water (2 mL) and 0.5 M NaCl (pH 2.5) (2 mL). The cartridge was washed with 1 mL of 0.5 M NaCl (pH 2.5) and SDZ was eluted with 2 mL of methanol:water (1:1 v:v). The eluate was evaporated to dryness under a stream of nitrogen, then redissolved with 500 µL of methanol:water (1:1 v:v) and transferred into vials for HPLC analysis.

Trimethoprim: the homogenate was diluted with 0.2 M CH_3COONa (20 mL) and then centrifuged at 1000 x ***g*** (15 min). The supernatant was cleared on SPE-C18 cartridge prewashed with CH_3OH (2 mL) and H_2O (2 mL). After the sample loading, the cartridge was washed with a 0.025 M KH_2PO_4-buffer solution (pH 4.5) (2 mL) and TMP was eluted with 2 mL of a mixture 0.025 M KH_2PO_4-buffer solution (pH 3.5):CH_3OH (10:90 v:v). The eluate was dried under vacuum (UNIVAPO, A.N. Kraupa), dissolved in 400 µL of 0.025 M KH_2PO_4-buffer solution (pH 4.5) and transferred into vials for HPLC analysis.

High-performance liquid chromatography

The chromatographic analyses of SDZ and TMP were performed by an HPLC system (Beckman System Gold equipped with UV-Diode array Beckman 168 detector and GOLD release 4.0 software (Beckman Inst. INC) and a reverse phase column ABZ (5 µm; 250x4.6 mm - Supelco) under the following conditions: mobile phases: see

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above; flow rate: 0.6 mL min^{-1}; injection volume: 50 µL; detection wavelength: 272 nm for SDZ and 230 nm for TMP. The total run time was 15 min.

Method validation

Linearity of the detector response was checked with the standard solution; the range was 0.05 to 2.0 µg mL^{-1} for SDZ and 0.02 to 1.0 µg mL^{-1} for TMP. Selectivity was evaluated by comparing the chromatograms of blank and spiked samples processed under the described conditions. Accuracy of the analytical method was assessed for SDZ and TMP by replicate analyses of samples fortified at 0.1-0.5-1 µg/g and was reported as percent recovery (rec%).

The inter-day precision of the method was checked, for each different tissue, by repetitively analysing tissues samples spiked at 0.1 µg/g for both the drugs and was expressed as coefficient of variation % (CV%).

The detection limit (LOD) was estimated by visual evaluation of the minimum level at which the two analytes can be reliably detected. The quantification limit (LOQ) was determined by the analyses of samples with known concentrations of analytes and by establishing the minimum level at which the single analytes could be quantified with accuracy and precision that fall within the range recommended by the EMEA (1996, 1998).

Calculation of withdrawal times

As suggested by the Committee for Veterinary Medicinal Products (EMEA 1995), withdrawal periods were set at the time point at which the concentrations of residues in all tissues for all animals fall below the respective MRL values. In order to compensate for the uncertainties of biological variability, the estimation of a safety span (10-30% of time period) was considered. A statistical model based on linear regression analysis was also used as an alternative approach to estimate withdrawal periods.

All residues of SDZ and TMP, which were below the LOQ as well as the MRL, were calculated and reported exactly. When residue values were also below the LOD, they were entered at half of the LOD value for calculation purposes. When all the data at a particular time point were lower than the LOD, the results were excluded from calculation. Regression models were fitted to the logarithms of the muscle, liver, kidney and skin/fat SDZ and TMP residue concentrations. Non-linearity of each regression model was assessed using the lack of fit test. Homogeneity of variances was assessed using Cochran and Barlett's methods, and the normality of residuals was checked using the Shapiro-Wilk test. One-sided upper tolerance limits (95%) with a 95% confidence were calculated from these regression models, based on the equation of Stange.

RESULTS AND DISCUSSION

The finalised analytical conditions gave retention times of 8.5±0.1 min and 7.2±0.1 min for SDZ and TMP, respectively. At these retention times, no interfering peaks were observed in the blank samples of the matrices examined. Representative HPLC chromatograms for sulphadiazine are reported in Figure 1 and for trimethoprim in Figure 2.

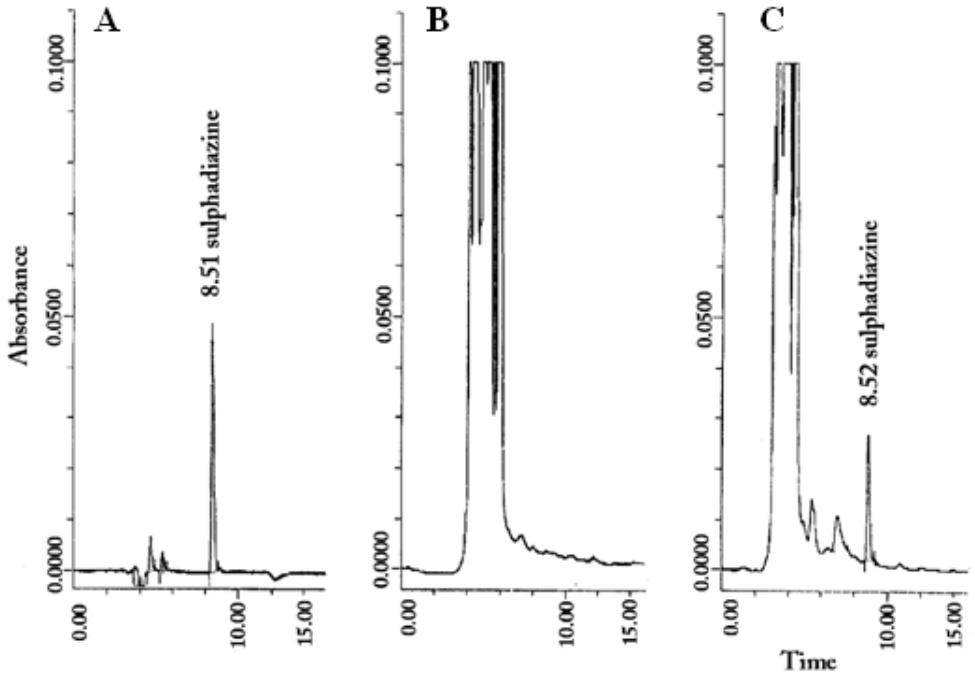

Figure 1. Representative HPLC chromatograms of (A) TMP standard (500 ng ml^{-1}), (B) a blank extracted broiler kidney sample and (C) an extracted broiler kidney sampled after treatment.

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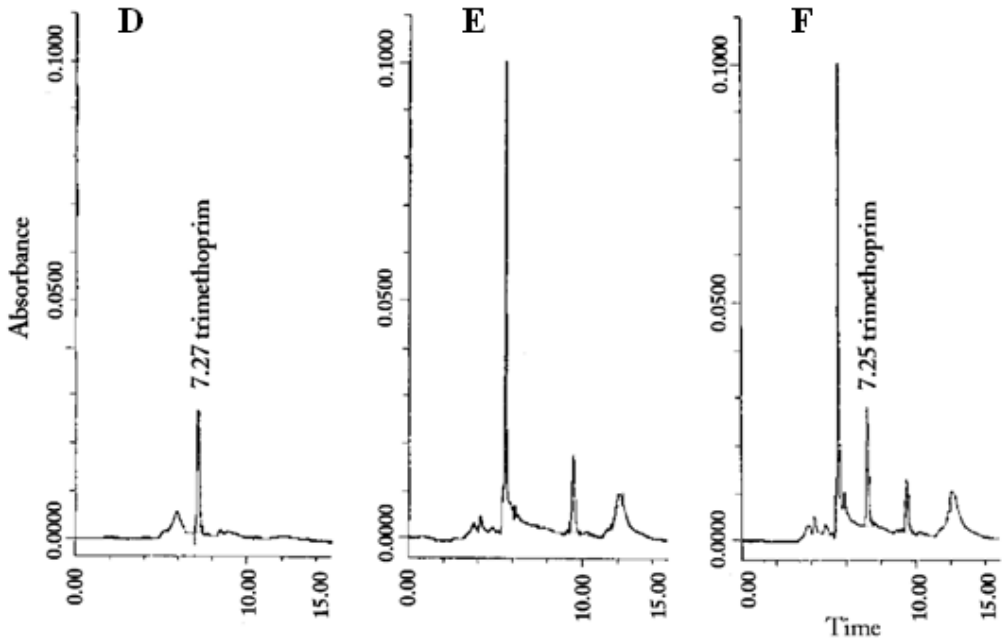

Figure 2. Representative HPLC chromatograms of (D) SDZ standard (500 ng ml^{-1}), (E) a blank extracted broiler kidney sample and (F) an extracted broiler kidney sampled after treatment.

The calibration curves for the two test antibacterial drugs were linear over the concentration ranges examined (i.e. SDZ 0.05-2.0 µg mL^{-1}; TMP 0.02-1.0 µg mL^{-1}) with correlation coefficients always greater than 0.999. The average recoveries (±SD) determined for each tissue and for both drugs at three different concentration levels, are reported in Table 1. Recoveries in the target tissues ranged from 76.79±0.74% (broiler) and 78.82±1.07% (pig) for SDZ and between 77.02±0.62% (broiler) and 80.49±1.15% (broiler) for TMP. The precision data expressed as CV%-values are given in Table 2.

Table 1 - The mean recovery (%), SD and CV (%) of sulphadiazine and trimethoprim from different spiked samples (n=6)

PIG									BROILER								
	Sulphadiazine				*Trimethoprim*					*Sulphadiazine*				*Trimethoprim*			
Tissue	mean	±	SD	CV%	mean	±	SD	CV%		mean	±	SD	CV%	mean	±	SD	CV%
Muscle	77.84	±	0.89	1.15	78.09	±	1.00	1.28	*muscle*	77.20	±	0.91	1.18	78.91	±	1.18	1.50
Liver	78.11	±	0.72	0.92	78.51	±	1.01	1.29	*liver*	77.84	±	1.11	1.43	78.22	±	1.36	1.74
Kidney	77.07	±	0.92	1.20	77.22	±	1.00	1.33	*kidney*	76.88	±	0.65	0.85	80.49	±	1.15	1.43
Fat	78.82	±	1.07	1.35	79.96	±	0.82	1.04	*fat*	76.79	±	0.74	0.97	77.02	±	0.62	0.80

Table 2 - Precision of sulphadiazine and trimethoprim determination at 0.1 µg g^{-1} in pig and broiler tissues (mean value and CV%; n=6)

PIG					BROILER				
	Sulphadiazine		*Trimethoprim*			*Sulphadiazine*		*Trimethoprim*	
Tissue	mean	CV%	mean	CV%	Tissue	mean	CV%	mean	CV%
Muscle	0.073	2.29	0.076	2.70	*Muscle*	0.075	3.44	0.076	1.35
Liver	0.073	1.12	0.076	1.97	*Liver*	0.075	3.60	0.075	3.37
Kidney	0.071	2.22	0.074	2.88	*Kidney*	0.085	3.17	0.078	2.41
Fat	0.073	2.83	0.075	2.16	*Fat*	0.083	1.91	0.077	1.44

In pigs, the inter-day precision ranged from 1.12% (liver) to 2.83% (skin/fat) for SDZ and from 1.97% (liver) to 2.88% (kidney) for TMP. In broilers, the inter-day precision ranged from 1.91% (skin/fat) to 3.44% (muscle) for SDZ and from 1.35% (muscle) to 3.37% (liver) for TMP. The LOD was defined for all tissues at 0.025 µg mL^{-1} for SDZ and at 0.020 µg mL^{-1} for TMP. For both drugs, a single LOQ value were validated for all tissues of the two animal species corresponding to one-half the MRL values (0.05 µg g^{-1} for SDZ and 0.025 µg g^{-1} for TMP). The mean values (±SD) of SDZ and TMP residues in target tissue of treated pigs and broilers are reported in Table 3 and Table 4, respectively.

One day after the intake of the last dose, SDZ and TMP tissue levels in pigs were higher than the corresponding MRLs in muscle, liver and kidney. In contrast, in skin/fat, while the SDZ residues were lower than the reference values (MRLs and LOQ), those for TMP were all higher than the defined residual limits.

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Table 3 - The mean (±SD) concentrations (µg g^{-1}) of sulphadiazine and trimethoprim in pig (4/group) tissues after oral administration

PIG						
Time after treatment (days)						
	Sulphadiazine			*Trimethoprim*		
Tissue	1	7	10	1	7	10
Muscle	0.172±0.015	0.068±0.018	-	0.099±0.015	0.036±0.004	-
Liver	0.234±0.031	0.063±0.006	-	0.171±0.010	0.035±0.006	-
Kidney	0.282±0.018	0.056±0,008	-	0.306±0.029	0.038±0.002	-
Fat	-	-	-	0.134±0.019	-	-

Table 4 - The mean (±SD) concentrations (µg g^{-1}) of sulphadiazine and trimethoprim in broiler (6/group) tissues after oral administration

BROILER								
Time after treatment (days)								
	Sulphadiazine				*Trimethoprim*			
Tissue	1	3	5	10	1	3	5	10
Muscle	0.102±0.011	-	-	-	0.038±0.006	-	-	-
Liver	0.187±0.054	0.081±0.006	0.058±0.002	-	0.066±0.017	-	-	-
Kidney	0.369±0.064	0.154±0.056	0.065±0.004	-	0.249±0.039	0.082±0.006	0.033±0.006	-
Fat	0.447±0.057	0.217±0.077	0.076±0.010	-	0.225±0.035	0.079±0.011	0.038±0.005	-

Sulphadiazine concentrations decreased rapidly during the following six days and reached levels lower than the MRLs in all target tissues. Nevertheless, it was detected over 0.05 µg g^{-1} (LOQ) in 3 muscles, 2 livers and 2 kidneys. Similarly, at the same time point, residual values of TMP were always lower than the MRLs, but higher than the LOQ in 2 muscle, 3 liver and 3 kidney samples. In skin/fat, TMP residues were all lower than the LOQ, on day 7.

Ten days after the end of the treatment both SDZ and TMP concentrations detected were lower than the LOQ in all tissues for all animals.

One day after the end of the treatment, SDZ residues in broilers were detected at values below the MRLs in only muscle from three animals. In all the other tissue samples, drug concentrations were higher than 100 µg g^{-1}. On the subsequent sampling time at day 3, the SDZ concentrations in all muscles were below the LOQ and in all livers below the MRLs. In contrast, the mean concentrations detected in kidney and skin/fat were still fairly high (0.154±0.06 µg g^{-1} and 0.217±0.08 µg g^{-1}). In these two last tissues, the SDZ residues did not fall below the MRLs until day 5 after withdrawal of treatment. At the following time point residual levels decreased also under the LOQ in all the analysed samples. At the first sampling point, TMP concentrations in broilers were lower than the LOQ in muscle from 4 animals and lower than the MRLs in the other two muscles and in one liver. The observed values in kidney and skin/fat were always over 0.050 µg g^{-1}.

After two days, drug concentrations decreased below 0.025 µg g^{-1} in all the muscle and in all the liver samples. TMP depletion in the other two tissues was slower and concentrations were below the validated LOQ in all tissues for all animals only at the last sampling point.

Sulphadiazine withdrawal periods calculated for muscle, liver, kidney and skin/fat were 8.4, 8.4, 8.4, 1.0 days and 3.0, 3.6, 6.0, 6.0 days in pig and broiler tissues, respectively. TMP withdrawal periods established for muscle, liver, kidney and skin/fat were 8.4, 8.4, 8.4, 7.0 days and 1, 3.6, 6.0, 6.0 days in pigs and broilers, respectively.

The analytical methods adopted in this study to evaluate the residual concentrations of SDZ and TMP in pig and broiler edible tissues had shown good selectivity, sensitivity and percentage of recovery from spiked tissues. The extraction and purification procedures of the two drugs from tissues appeared simple and show a good repeatability. The samples were processed in a short time and without hazardous wastes. The limits of quantitation (LOQ) appeared suitable for the residue depletion studies. The experimental protocol for the residue depletion study simulated the field conditions. The variation of the feed consumption and, as a consequence, of the oral dose of sulfadiazine and TMP during the treatment was within acceptable limits (mean CV: 4.25% for pigs and 8.21% for broilers, respectively).

The use of the statistical linear regression model to estimate the withdrawal times requires that some regression assumptions such as homogeneity of variances of the $\log_e$-transformed data on each slaughter day, linearity of the $\log_e$-transformed data versus time and a normal distribution of the errors are valid. Our residual data detected in pig and broiler tissues did not always satisfy one or more of these conditions (Table 5). For this reason, the withdrawal periods established correspond to the time points at which the concentration of residues in all tissues for all animals fell below the respective MRLs plus a 20% safety span. When all observations were below the LOQ, this safety span value was not applied. Garwacki et al. (1996) following administration to pigs of a medicated SDZ/TMP feed (30 mg kg^{-1} bw/6 mg kg^{-1}bw) for five days, found that both drugs were rapidly eliminated. Five days after the treatment, SDZ was not detected in any tissue, whereas TMP was present at concentrations of 0.01 µg g^{-1} (muscle), 0.02 µg g^{-1} (liver) and 0.03 µg g^{-1} (kidney).

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Table 5 - Results of statistical linear regression model applied for the withdrawal period evaluation in pigs and broilers after oral administration of sulphadiazine and thrimetoprim

Sulphadiazine						
		F-test	**Cochran-test**	**Barlett-test**	**Shapiro/Wilk**	**WT**
	Tissue	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.10)	(days)
Pig	Muscle	p>0.05	0.01<p<0.05	manually performed[2]	p>0.10	-
	Liver	0.05>p>0.025	p>0.05	manually performed	0.05>p>0.02	-
	Kidney	p<0.025	p>0.05	manually performed	0.10>p>0.05	-
	Fat[1]	-	-	-	-	-
Broiler	Muscle	p<0.025	p>0.05	p<0.01	p>0.1	-
	Liver	p>0.05	p>0.05	p>0.05	p>0.1	5.3
	Kidney	p>0.05	p<0.01	p>0.1	p>0.1	-
	Fat	p>0.05	p<0.01	0.05>p>0.025	p>0.1	-
Thrimetoprim						
		F-test	**Cochran-test**	**Barlett-test**	**Shapiro/Wilk**	**WT**
	Tissue	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.05)	(n.s. p>0.10)	(days)
Pig	Muscle	p>0.05	p>0.05	manually performed[2]	p>0.10	8.3
	Liver	p>0.05	p>0.05	manually performed	p>0.10	7.9
	Kidney	p>0.05	p>0.05	manually performed	p>0.10	8.6
	Fat[1]	p>0.05	p<0.01	manually performed	p<0.01	-
Broiler	Muscle	-	-	-	-	-
	Liver	p>0.05	p<0.01	p<0.01	0.10>p>0.05	-
	Kidney	p>0.05	p<0.01	p<0.01	p<0.01	-
	Fat	p>0.05	p>0.05	p>0.05	p>0.10	-

[1]not statistically analyzed because residual concentrations were below the corresponding MRL and LOQ at the first time point. [2]because each group number <5

Eight days after the last dose the drug was not detected in any tissue. On the basis of their results, the authors proposed a withdrawal time not less than 5 days for the formulation used in pigs. In contrast with these findings, one day after the last intake, we found levels of SDZ higher than those of TMP in the muscle and liver and lower in the kidney. Sulphadiazine was detected until 7 days after the last intake. In addition, TMP residual depletion was more prolonged and values detected at this time point were close to the MRL. These differences could be related to physiological or environmental conditions, particularly to the free access to feed, with a consequent different daily intake of the two drugs during the present residual depletion studies. Dagorn et al. (1992), after a SDZ/TMP combination administered at 20/4 mg kg^{-1} b.w./daily in broilers via drinking water for 4 days, observed a more rapid decrease of TMP. Forty-nine hours after the end of the medication, TMP residue concentrations were detected only in skin with values close to 0.05 µg g^{-1}, whereas SDZ skin concentrations reached mean values of 0.14±0.04 µg g^{-1}. The calculated withdrawal time was 7.28 days. In contrast, in our study the SDZ withdrawal period gave evidence of a similar rapid decrease of this drug, notwithstanding different levels detected on the first time point in the liver, kidney and skin/fat.

CONCLUSION

To ensure safe residue levels in all target tissues, withdrawal periods of 8.6 days and 6.0 days should be applied to pigs and broilers treated with 700 ppm of SDZ and 140 ppm of TMP and with 300 ppm of SDZ and 60 ppm of TMP in feed, respectively.

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MARKETTING SITUATIONS OF LIVESTOCK FEEDS IN WELMERA AND DENDI WEREDA OF WEST SHOA ZONE, ETHIOPIA

R. MESFIN*, A. TESFAYE

Ethiopian Institute of Agricultural Research, Holetta Research Center,

*E-mail: Y_takele@yahoo.com

ABSTRACT: *The paper explains the status of livestock feed resources and market situations in Welmera and Dendi weredas of West Shoa Zone, Ethiopia. The objective of the survey was to assess the potentials and constraints of feed resources and related marketing practices and suggest appropriate intervention options to overcome the constraints. Majority (76%) of the interviewed farmers have faced shortage of livestock feeds. The diminishing trend of grazing land from time to time, roughage, concentrate feeds are the factors contributing to feed shortage. Moreover, the increasing trend in selling price of hay and concentrate feeds aggravates more to the problem. This situation is limiting livestock productive in the highlands of Ethiopia. Under this condition, farmers purchase feeds to both local and crossbred animals. The purchased feeds include: hay, straw, grazing area, oilseed cakes, wheat bran and wet grass. Among these, the grazing area purchased takes the highest (52%) proportion. Farmers and traders participate in purchasing of livestock feeds. The proportion of farmers that purchase feeds is higher (30%) than that of the traders (1%). To alleviate the problems related to shortage of livestock feeds and decline of animal production and productivity, rearing of improved crossbred dairy cattle under intensive management and forage/fodder development and feeds conservation schemes should be promoted in a wider scale. Considering the ever-increasing price of feeds, there is a need to shift from purchased commercial feeds to the use of farm produced feed resources.*

Key words: *Farmers, Grazing Land, Roughage, Concentrate Improved Forage*

ORIGINAL ARTICLE

INTRODUCTION

Agriculture in Ethiopia is indispensable component of rural livelihoods. Livestock has direct contribution to human food, draft power and, manure. Market oriented livestock production contributes to income generation, economic stability and serves for securing foreign currency (Mirjam, 1998). Moreover, the pastoral community depends entirely on livestock for their livelihood (Little et al., 2001; Barret et al., 2003).

Though, the country has great potential for increasing livestock production, both for local use and export market, expansion was constrained by in adequate nutrition. Feed is the most important input and is an essential prerequisite for sustained livestock production. Livestock feeding in Ethiopia is based on grazing mainly on natural pasture and fallow lands which accounted close to 26,601606 tones DM. This is augmented with feeding crop residues, which estimated to 31,300,146 tones. Stubble grazing following crop harvest also accounts for about 22% of the total feed supply (CSA, 2001). Due to the rapidly increasing human population and expansion of cropping in to grazing areas, the importance of natural pasture and fallow land as source of feed is decreasing from time to (Adugna, 2009). In the Ethiopian highlands, the feed requirement of indigenous livestock population is estimated to be 55 million tons of dry matter (DM). This is much higher than what is available in the real situation, which is estimated to be 40.1 million tons DM (Betre, 2000).

There is seasonal variation in feed availability and quality. During dry season, livestock feed is in short supply and is also of poor quality. During this, residues from cereals are the main source of roughage, which are low in protein and poor digestibility (Alemayehu, 2002; Tessema et al., 2002; Tesfaye et al., 2009).

Feed has become a marketable commodity in different parts of the country, particularly around towns and big cities. The type of feeds marketed in different places is very diverse and in most cases include roughage and concentrate (Adugna, 2009). Attractive market and marketing system determines the development of animal agriculture and encourage producers to produce more. The existing production and marketing of livestock feed can be improved through implementation of appropriate interventions through designing appropriate research strategy. Assessment of the situation and dynamism of livestock feeds system and analysis of critical constraint is important to point out problems of farmers, the opportunities that exist within the farming system and to design relevant

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research strategy. The objectives of this study was therefore to assess the marketing practices of livestock feed resources, to identify constraints and opportunities related to marketing of livestock feeds and to suggest appropriate intervention options to overcome the constraints.

MATERIALS AND METHODS

Place of the study

The study was carried out in two weredas namely, Welmera and Dendi. Welmera wereda is located in Oromiya region, West Shoa Zone along the Addis Ababa-Ambo road about 40 km West of Addis Ababa. Geographically the wereda is situated between 09^0 03' latitude 38^0 30' longitudes. The altitude of the wereda ranges from 2060 to 3380 m.a.s.l. The rainfall pattern of the wereda follows a pattern of bi-modal. Most of the rain falls during the main rainy season (June to September). Short rains (Belg) commence from January to February and extend up to May.

Dendi wereda is similarly located in Oromiya region, West Shoa Zone along the Addis Ababa-Ambo road about 70 km west of Addis Ababa. The altitude ranges from 1500 to 3270 m.a.s.l. The place is experienced bimodal rainfall: the short rainy season is during March and April followed by long rainy season during June to September. Annual rainfall ranges from 0.7 – 265 mm, in the upper and lower Kola. The dominant soil type is black soil (vertisol).

Respondents

The interview was carried out on a total of 228 farmers in Welmera and Dendi Weredas. Eighty farmers were interviewed from Welmera and the rest (148) of them were from Dendi wereda.

Method of data collection and analysis

Data was collected based on a survey using questionnaires. Farmers were systematically interviewed based on the prepared questionnaire. The collected data was organized and analyzed using (SPSS, 2003). Qualitative data were analyzed based on descriptive statistics and the quantitative data were analyzed using comparison of means and t-test.

RESULTS AND DISCUSSIONS

Marketing of animal feeds and grazing area

Majority (60%) of the interviewed farmers in both weredas reported that they purchase animal feed (Table 1). The interviewed farmers purchase all types of feeds. The feeds purchased include hay, straw, grazing area, oil seed cakes, wheat bran and wet grass. Majority (51.6%) of the interviewed farmers mainly purchase grazing area. Oil seedcakes were the second major (30.7%) type of feed that farmers have been purchasing to feed their animals. Were as, wheat bran was ranked the third (18.5%). Commercial feed (concentrate) are either less available or too costly to farmers. However, minor (3.5%) proportion of the interviewed farmers purchases straw. Farmers have the access of owing crop residues because they cultivate food crops every year (Table 2).

Table 1 - Do you purchase animal feed by Wereda

Farmers purchase animal feed	Wereda					
	Welmera		Dendi		Overall sample	
	N	%	N	%	N	%
Yes	48	60	88	60	136	60
No	32	40	58	40	90	40

Table 2 - Type of Livestock feed purchased by Woreda

Type of feed	Wereda		
	Welmera (%)	Dendi (%)	Overall sample (%)
Hay	33.8	12.5	21
Straw	5	2.5	3.5
Grazing area	36.3	60.6	51.6
Oil seed cake	38.8	25.4	30.7
Wheat bran	41.3	3.3	18.5
Wet grass	26.3	11.8	17.4
Total	181.5	116.1	142.7

Place of feed purchase

Different types livestock feeds were marketed in different places /markets in villages depending on availability and accessibility. About 56.8% and 56% of the interviewed farmers that have been purchasing hay and wheat bran respectively were from any place /site where available. Whereas, majority of the interviewed farmers (91.6%) the access of purchasing straw from neighboring farmers. About 42.9% of the interviewed farmers that have been purchasing grazing area where from farmer's field in the surrounding. Almost all of the interviewed farmers (100%) that have been purchasing oil seed cakes were from the neighboring (Ginchi) town (Table 3).

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Table 3 - Place of feed purchase of both weredas						
Place of purchase	Over all sample (%)					
	Animal feeds and grazing area					
	Hay	Straw	Grazing area	Oilseed cake	Wheat bran	Wet grass
Neighboring	-	91.6	-	-	-	-
Village	-	-	-	-	-	-
Any area/site where available	56.8	-	-	-	56	-
Ginchi	-	-	-	100	-	-
Trader	-	-	-	-	-	-
Farmers field in the surrounding	-	-	42.9	-	-	-
Farmers field away	-	-	-	-	-	-
Keba	-	-	-	-	-	-
Soko	-	-	-	-	-	-
Awumara	-	-	-	-	-	-
Abebe	41	-	-	-	-	-
Mumea	-	-	-	-	-	-

Purchase of feeds for different breeds of animals

Forty four percent (44.2%) of the interviewed farmers have been purchasing hay to feed local animals. Whereas, the proportion of farmers that have been purchasing hay to feed crossbred animals was by far less than (3.2%) that of the farmers purchased hay to feed local animals (44.2%). Fewer proportion (8.4% and 1.2%) of the interviewed farmers purchase straw to feed local and crossbred animals respectively. In addition, 69% and 2.5% of the interviewed farmers purchase grazing area for local and crossbred animals respectively. With regard to concentrate feeds, 55.9% and 3.6% of the farmers purchased oilseed cakes to supplement local and crossbred animals respectively. Similarly, 44% and 4.8% of the interviewed farmers have been purchased wheat bran to supplement local and crossbred animals respectively. About 38% and 21% of the interviewed farmers have been purchasing wet grass to feed local and crossbred animals respectively (Table 4).

Regardless of the breed of an animal to be fed, the highest proportion (71.2%) of the interviewed farmers has been purchasing grazing area. This indicates that grazing area is the most limiting factor for rearing animals in the highlands. Oil seed cakes are the second most feed type that have been purchased (59.5%) to supplement both local and crossbred animals. Because of its abundance in the highlands, oil seed cakes retain the second rank among the purchased concentrate feeds. Similarly, 58.9% of the interviewed farmers have been purchasing wet grass to feed both local and crossbred animals. This implies that during rainy season in the highlands, there is shortage of feeds and animals concentrate in areas that are not suitable for croplands. Close to fifty percent (48%) of the interviewed farmers purchase wheat bran as concentrate feed to supplement both local and crossbred animals. The proportion of farmers that have been purchasing wheat bran was less than that of the farmers purchasing oil seed cakes. This is because oil seed cakes are more abundant than wheat bran. This is because flour milling factories in the survey area are less available. Close to flirty percent (47.4%) of the interviewed farmers also purchased hay to feed both local and crossbred animals. This indicates that farmers in the highlands have a problem of shortage of roughage feeds. This is related to the limitation of adequate and fertile grazing area that can grow forage feeds that can satisfy the feed requirement of animals. However very few proportion (8.6%) of the interviewed farmers purchases straw to crossbred animals. Since all farmers in the highlands cultivate food crops, crop residues including straws and stoves are abundant throughout the year. That is why very less number of farmers' purchases straw to feed animals. This implies that the amount of straw produced by some farmers is not adequate to feed their animals. This may be related to scarcity of cropland owned by individual farmers. However, farmers should not feed crop residues as it is. They should either treat them or supplement to upgrade their nutritive value (Table 4).

Table 4 - Purchase of feeds for different breed of animals in both weredas			
Feed types	Over all sample farmers purchased feed and grazing area (%)		
	breeds of animals		
	Local animals	Crossbred animals	Total
Hay	44.2	3.2	47.4
Straw	8.4	1.2	8.6
Grazing area	68.7	2.5	71.2
Oil seed cake	55.9	3.6	59.5
Wheat bran	44	4.8	48
Wet grass	37.9	21	58.9

Reasons for not buying Livestock feed

About 48% of interviewed farmers did not purchase livestock feeds. Having enough feed,, lack of cash, unavailability of feed for sale, expensiveness of the feeds and others were the possible reasons for not buying livestock feeds. Farmers interviewed from Welmera wereda have been buying more animal feeds as compared to farmers from Dendi (Table 5).

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Table 5 - Reasons for not buying Livestock feed by wereda			
Reasons	Wereda		
	Welmera (%)	Dendi (%)	Overall sample (%)
Having enough feed	25	75	44.7
Lack of cash	12.5	18.5	14.9
Unavailability of feed for sale	1.3	5.6	3
Expensiveness	11.3	3.7	8.2
Other	1.3	1.9	1.5
Total	51.4	104.7	72.3

Problems related to Livestock feed

About 76% percent of the interviewed farmers in both weredas reported that there was shortage of animal feed. Because of this reason, majority of the farmers have forced to purchase animal feed (Table 6).

Table 6 - Problems related to Livestock feed by wereda			
Reasons	Wereda		
	Welmera (%)	Dendi (%)	Overall sample (%)
Yes	75	76.4	75.9
No	25	23.6	24.1

Source for purchase of Livestock feed

With regard to the source of feed purchase, majority of the interviewed farmers (69%) have been buying livestock feeds from other farmers. This indicates that other class of the society have not yet been take part in doing business on the agricultural sector Whereas, about 29% of the interviewed farmers have been purchasing feed from traders. About 4% of the interviewed farmers have been purchasing from any seller. As we compare the two weredas interns of source of feed purchase, farmers from Welmera wereda purchase animal feed from trader than those farmers from Dendi wereda. Whereas farmers from Dendi wereda have the access of purchasing animal feed from other farmers. This is because as compared to Dendi wereda, Welmera wereda is more closer to the cities Holetta and Addis Ababa. It is easier for Businessmen to run agricultural trading to the nearest city so as to minimize the transport cost (Table 7).

Table 7 - Source for purchase of Livestock feed by wereda			
Seller	Wereda		
	Welmera (%)	Dendi (%)	Overall sample (%)
From trader	43.8	17	29.3
From other farmer	46.3	88.3	69
From any seller	6.3	2.1	4
Total	96.4	107.4	102.3

For whom do farmers sell Livestock feed

For the interviewed farmers who have been selling livestock feeds, they have been mainly selling feeds to other farmers. As compared to farmers from Welmera, those farmers from Dendi wereda have the opportunity of selling animal feeds to other farmers. This implies that, there was no trader in Dendi wereda that can purchase livestock feed. This may be related to the closeness of Welmera wereda to cities like Holetta and Addis Ababa. As compared to farmers from welmera, farmers from Dendi have mainly the access of selling feeds. This may be related to the access of producing adequate feeds for farmers in Dendi Wereda than in welmera Wereda (table 8).

Table 8 - For whom do you sell Livestock feed by wereda?			
Buyer of Livestock feed	Wereda		
	Welmera (%)	Dendi (%)	Overall sample (%)
To trader	1.3	0	1.1
To other farmer	21.3	90.9	29.7
Total	22.6	90.9	30.8

Problems related to market of livestock feed

There have been many problems related to livestock feed. High price, poor quality and low price of animal feeds were the possible problems. Among which, high selling price of feed was mainly (38.2%) affecting market of livestock feeds. Poor quality feeds were the second problem (15.8%), affecting market of Livestock feed (table 9).

Table 9 - Problems related to market of livestock feed * Woreda			
Problem	Wereda		
	Welmera (%)	Dendi (%)	Overall sample (%)
High price	36.3	39.8	38.2
Poor quality	35	0	15.8
Low price	7.5	1	4
Total	78.8	40.8	58

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Area of land used for different purposes

Among the areas of land used for different functions, cultivated land constitutes the largest (2.47). Fallow land constitutes the second in area coverage (0.63 ha) and grazing land comes third (0.52 ha). Relatively very small area, 0.05 and 0.01 ha were allocated for vegetable land and tree land respectively. Alemayehu (2005) observed similar result in that tree land constitutes the smallest proportion of the land allocated for different uses. It was also similarly reported that greater proportion (3.2 ha) of the land was allocated for cultivation of crops and only 1 ha and 0.4 ha of land were allocated for grazing and other areas respectively. It was observed that the proportion of land allocated for cultivation of crops, for grazing and so on was declining from year to year. This has become the main reason for shortage of feed and decline of livestock holding and productivity. Because grazing and browsing account the major (88%) portion of the total feed supply in Ethiopia (Zelalem, 1999). In comparing the two weredas, the average land holding of farmers in Welmera wereda was greater than that of the farmers in Dendi. (Figure 1).

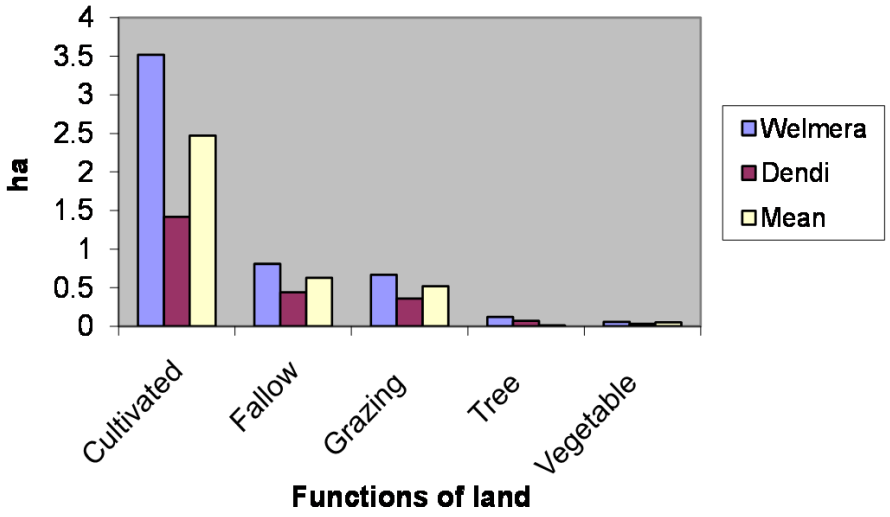

Figure 1 - Average prices of concentrate feeds and hay in different years

The increase in price of animal feeds including hay and different concentrate ingredients starting from the year 1990 to 1999 was gradual and was stable. The increase in price of feeds from the year 1999 to the year 2000 was in abrupt condition. As compared to the year 1999, the price of concentrate feed has reached in to 2-3 folds of the pries in the year 1999. The abrupt increase in the year 2000 was more for concentrate feeds than the price of hay. Among the price of different concentrate ingredients the price increase of noug cake was the highest. In recent times grain of noug seed has got great export demand. Due to this, the price of noug cake in the domestic market has become too expensive. Next to this the price of other concentrate ingredient feeds like wheat bran and wheat middling has increased in accelerated trend in the year 2000 (Figure 2).

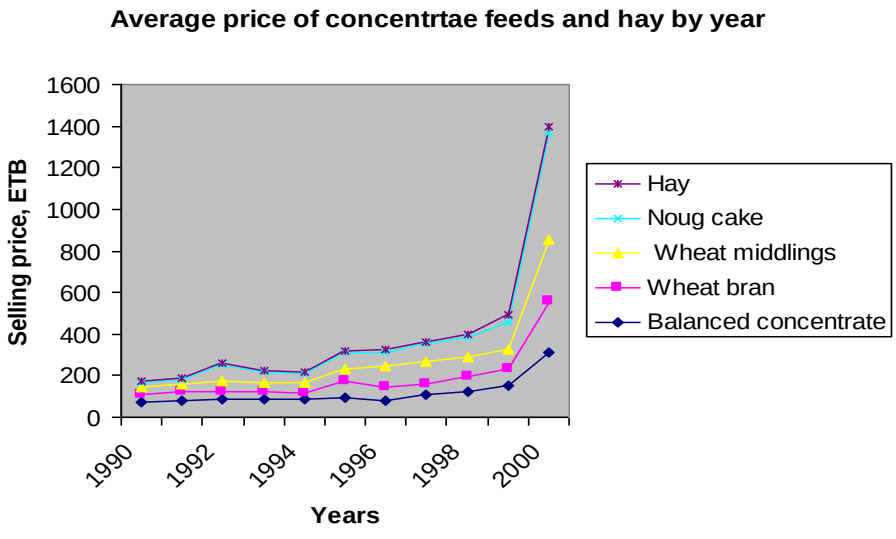

Figure 2 - Average price of concentrate feeds and hay

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CONCLUSIONS

The survey results revealed that majority of the interviewed farmers have faced feed shortage problems. As a result they are forced to purchase livestock feeds. The major problems associated to feeds and marketing are high purchased price and poor quality. Among the areas of land used for different functions, land allocated for crop cultivation constitutes the largest. The proportion of land allocated for fallowing, grazing, vegetable production and tree growing goes respectively in decreasing order. Price of livestock feeds is accelerating with time.

RECOMENDATIONS

To alleviate the problems of feed shortage and decline of livestock productivity, intensive handling of improved crossbred dairy cows, forage development and feeds conservation schemes should be promoted in wider scale. Considering the ever-increased price of feeds, there is a need to shift from purchase commercial feeds to use of farm produced feed resources. Policy considerations focusing to development of livestock feed supply is required in Ethiopia.

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ADDITION OF PROTEIN SOURCES FOR CALVES SUPPLEMENTED WITH HIGH MOISTURE SORGHUM GRAIN SILAGE GRAZING LOW-QUALITY PASTURES

P. ROVIRA*

Instituto Nacional de Investigación Agropecuaria (INIA), Ruta 8 km 281, CP 33.000, Treinta y Tres, Uruguay

*E-mail: provira@tyt.inia.org.uy

ABSTRACT: *Three experiments were conducted to determine the effect of protein addition to high moisture sorghum grain silage (HMS) daily supplemented to calves at a rate of 1% of body weight (BW) grazing low-quality pastures. In exp. 1 addition of sunflower expeller or a protein ration to increase crude protein (CP) of HMS from 7.1% to 12% increased average daily gain 56% compared with calves fed only HMS (0.39 and 0.25 kg/a/d, respectively). Calves supplemented with protein sources were more efficient than calves supplemented only with HMS as feed conversion numerically decreased from 6.0 (HMS) to 4.5 (HMS + sunflower expeller) and 4.1 (HMS + protein ration). In exp. 2 CP of HMS (9.1%) was increased to 15.5% by adding sunflower expeller, urea or combination of both. Protein supplementation increased ADG and final BW (0.20 kg/a/d and 196 kg) compared with only HMS (0.03 kg/a/d and 176 kg). Protein source had no effect on animal performance. In exp. 3 CP concentrations in the supplement had a significant effect on ADG when increased from 8.9 to 16.1% (0.32 and 0.50 kg/a/d). Performance of calves fed either 16.1% or 20.8% CP supplements did not differ possibly because energy was becoming the limiting factor at the highest CP concentration level. Rib eye area and fat thickness were not affected by treatment although supplemented calves registered 7% and 10% greater values in those variables, respectively, than un-supplemented animals at the end of the experiment. The addition of protein sources to HMS increased performance of calves grazing low-quality pastures.*

Key words: *Calves, Pastures, Sorghum Silage, Protein Addition, Supplementation*

ORIGINAL ARTICLE

INTRODUCTION

Land use has changed in traditional livestock operations driven by an increase in economic returns of agriculture, dairy and forestry sectors reducing the total land under beef farming. To achieve acceptable levels of production and keep the sector competitive livestock producers supplement grazing cattle. In recent years it has become more popular the use of high moisture sorghum grain silage (HMS) as supplement due to the increased problems of availability and price variability of dry feed grains. This supplement is defined as the grain harvested with 22–30% moisture, ground and conserved under conditions of anaerobiosis in silo bags in the producer's own farm (Fassio et al., 2009).

The use of HMS in intensive beef grazing systems based on high-quality pastures (i.e. ryegrass, legumes) increases animal performance due to the high level of soluble carbohydrates available for rumen fermentation (Alvarez et al., 2001; Abdelhadi et al., 2005). However, when HMS are offered to animals grazing low-quality pastures ruminal ammonia concentrations are very low leading to decrease microbial crude protein synthesis and growth (Chase and Hibberd, 1987; Sanson and Clanton, 1989; Sanson et al., 1990; Bodine et al., 2000). This situation is becoming more common as beef farming depends more on extensive low-quality pastures due to the advance of dairy and agriculture in more fertile lands. The addition of an adequate amount of ruminally degraded protein can alleviate the deficit of nitrogen believed to result from feeding high-energy supplements with low-quality pastures increasing beef cattle performance (Del Curto et al., 2000). The current serie of studies were designed to determine whether the performance of calves grazing low-quality pastures and supplemented with HMS could be improved by different sources of supplemental protein.

MATERIALS AND METHODS

Three independent experiments were conducted at the National Institute of Agricultural Research (INIA) Experimental Station located in eastern Uruguay (latitude 33' 14'S, longitude 54' 15'W) during the period 2009-

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2011. The Meat and Wool Research Program of INIA approved all operational and ethical procedures involving animals. At the beginning of each experiment calves were treated for internal and external parasites. Health status of the cattle was recorded periodically based on fecal egg counts to determine further treatments. In each year calves 8-month old continuously grazing low-quality natural pastures (2.4-3-2 calves/ha) were assigned to different supplementation treatments. Supplements were offered once daily from Monday to Sunday at 09.00 h at a level of 1.0% of body weight (BW) on a dry matter (DM) basis.

In exp. 1 the study was carried out from 1 July to 8 October 2009 (100 days). Sixty four Hereford x A. Angus calves averaging 188 ± 14 kg of body weight (BW) were randomly assigned into 4 supplementation treatments without replication. Treatments were: T1) control without supplementation, T2) 100% high moisture sorghum grain (HMS), T3) 78% HMS + 22% sunflower expeller (SE), T4) 78% HMS + 22% commercial protein supplement (CPS). Composition of supplements were (DM basis): 7.1% CP and 12.8% ADF (HMS); 29.6% CP and 22.8% ADF (SE); 31.1% CP and 32.8 ADF (CPS). The CPS had a maximum level of urea of 6.5% according to label instructions.

In exp. 2 the study was carried out from 26 May to 14 September 2010 (111 days). Sixty four Hereford x A. Angus calves averaging 172±14 kg of body weight (BW) were randomly assigned into 4 supplementation treatments without replication. Treatments were: T1) 100% high moisture sorghum grain (HMS), T2) 75% HMS + 25% sunflower expeller (SE), T3) 86.7% HMS + 12.1% SE + 1.2 urea (U), and T4) 97.7% HMS + 2.3% U. Composition of supplements were (DM basis): 9.1% CP and 6.3% ADF (HMS); 34.7% CP and 32.7% ADF (SE); and 285% CP (U).

In exp. 3 the study was carried out from 24 June to 4 October 2011 (102 days). Sixty Hereford x A. Angus calves averaging 143±13 kg of body weight (BW) were randomly assigned into 5 supplementation treatments without replication. Treatments were: T1) control without supplementation, T2)100% high moisture sorghum grain (HMS), T3) 97% HMS + 3% slow released urea (SRU), T4) 94% HMS + 6% SRU, and T5) 75% HMS + 6% SRU + 19% sunflower expeller (SE). Composition of supplements were (DM basis): 8.9% CP and 5.9% ADF (HMS); 35.0% CP and 25.1% ADF (SE); 140% CP and 1.35% ADF (SRU).

Analytical procedures and measurements were similar for all three experiments. Pasture height and availability were registered for each treatment every 28 days by clipping 10 random 0.1 m^2 quadrants in each treatment at ground level. Forage samples were dried in a forced-air oven at 60ºC during 48 hours to estimate % DM and forage availability. The nutritive value of feeds and pastures was estimated following the standard procedures performed in the Animal Nutrition Laboratory of INIA and reported by Fassio et al. (2009). Each year the sorghum grain was harvested at physiological maturity (28-32% moisture), grounded and stored in the absence of oxygen in pressed-silo bags (60 m of length) using a commercial grain bagger machine. Bags were hermetically sealed for at least 21 days before feeding.

Protein supplements were thoroughly hand-mixed with the high moisture sorghum grain when it was extracted daily from the silo bag and then the combined feeds were delivered to the animals in the feed troughs. The quantity of supplement provided per animal increased gradually during a 2 weeks habituation period until it reached the level of 1% of BW per day. This period of adaptation was not included in the analysis of data. Animals were weighed early in the morning without previous fasting every 14 days to adjust the amount of supplement to be delivered in each treatment. Fasted BW was registered every 28 days to estimate average daily gain (ADG). In addition, calves were evaluated for rib eye area and subcutaneous fat at the *Longissimus dorsi* muscle between the 12^{th} and 13^{th} rib by the use of ultrasound performed by trained personal during experiments 2 and 3. Feed efficiency of supplemented treatments was calculated as the kg of supplement (DM basis) per kg of added gain above the performance of control calves.

Variables of body weight and average daily gain were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment means were compared when a significant ($P<0.05$) F-test was observed using LSM test.

RESULTS AND DISCUSSION

In each experiment treatment had no effect on herbage mass, sward height or chemical composition of pasture so data is presented averaged over treatments (Table 1). Natural pastures species are the main source of feed for ruminants in Uruguay all year around. Uneven seasonal growth and availability of pastures can be compensated through the transfer of herbage mass from high (spring) to low (winter) growing seasons but at expenses of lower quality. In each year average pasture allowance was high (>2,000 DM kg/ha) but low in nutritive values, as reflected by its low CP concentration, high proportion of dead (dry) forage and high concentration of neutral detergent fiber (NDF). Animal intake was limited by the low quality of forage as NDF is negative associated with forage intake (Holecheck and Vavra, 1982) and the low CP concentration supported the concept that protein supplementation is a major factor in natural pastures grazing by growing cattle.

Exp. 1 evaluated the use of two protein sources, either sunflower expeller (29.6% CP) or a protein commercial supplement (31.1% CP), mixed with high moisture sorghum grain silage (HMS) to increase CP concentration from 7.1% to 12.0%. Average daily gain (ADG) was increased ($P<0.05$) by overall supplementation compared with the control group (0.34 and -0.08 kg/a/day, respectively) (Table 2). Calves without supplementation were 18% lighter ($P<0.05$) at the end of the experiment compared with supplemented animals (182 and 222 kg, respectively) as the intake of low-quality pasture does not provide sufficiently digestible energy to meet the animals' maintenance energy requirements (Hunter and Vercoe 1987; Dicker et al., 2001). There was a greater response in ADG in treatments with protein sources compared with only HMS supplementation (0.39 and 0.25 kg/a/day, respectively).

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No difference (P>0.05) was detected between sunflower expeller (T3) and the commercial supplement (T4) as protein source for ADG. This result confirms that supplementation with protein is necessary to optimize production in ruminants consuming low-quality forages (Bohnert et al., 2002; Moss et al., 2003). The improvement in animal performance may be related to increased concentration in ruminal ammonia as a result of greater nitrogen supply resulting in a more favorable environment for rumen microbes and increased forage utilization (DelCurto et al., 1990; Ludden et al., 1995; Koster et al., 1996). The amount of increase in dry matter intake seems to be associated with the level of protein in the forage as well as the maturity of the forage (Kunkle et al., 1999). Calves supplemented with high moisture grain plus protein sources were more efficient than calves supplemented only with high moisture grain as feed conversion numerically decreased from 6.0 (T2) to 4.5 (T3) and 4.1 (T4).

Table 1 - Mean (± SD) pasture allowance, sward height and chemical composition (%DM) of the natural pasture for each year

Item	Year		
	2009	2010	2011
Pasture allowance, DM kg/ha	2,649±1,124	2,827±1,419	2,235±926
Sward height, cm	10.8±4.9	10.7±5.4	6.0±2.3
Green to dead forage ratio	36:64	39:61	28:72
Crude Protein (CP), %	7.8±1.5	6.4±0.5	7.9±1.3
Acidic Detergent Fiber (ADF), %	44.9±1.6	50.7±2.0	46.2±2.8
Neutral Detergent Fiber (NDF), %	74.3±2.2	72.0±1.3	64.6±5.6

Table 2 - Average daily gain (ADG) and feed efficiency (FE) for calves grazing natural pastures and fed high moisture sorghum grain with or without different protein sources (media ± SEM)[1]

Item	Treatment [2]				Prob.
	T1	T2	T3	T4	
% CP in supplement[3]	-	7.1	12.0	12.0	-
Initial weight, kg	189[a]±5	182[a]±4	192[a]±4	190[a]±3	0.25
Final weight, kg	182[a]±5	206[b]±5	227[c]±4	232[c]±4	<0.05
ADG, kg/a/day	-0.08[a]±0.03	0.25[b]±0.02	0.36[c]±0.03	0.42[c]±0.04	<0.05
FE[4]	-	6.0	4.5	4.1	-

[1] Means within a row with different superscripts differ (P<0.05). [2] T1: control without supplementation, T2: 100% high moisture sorghum grain (HMS), T3) 78% HMS + 22% sunflower expeller (SE), T4) 78% HMS + 22% commercial protein supplement (CPS). [3] CP: Crude Protein; supplement offered daily at 1% of body weight (DM basis). [4] Kg of supplement (DM basis) per kg of added gain above the performance of control calves

Table 3 - Average daily gain (ADG), rib eye area (REA) and fat thickness (FT) for calves grazing natural pastures and fed high moisture sorghum grain with or without different protein sources (media ± SEM)[1]

Item	Treatment [2]				Prob.
	T1	T2	T3	T4	
% CP in supplement[3]	9.1	15.5	15.5	15.5	-
% CP from urea	0	0	22	42	-
Initial weight, kg	172[a]±4	172[a]±3	172[a]±4	172[a]±3	0.92
Final weight, kg	176[a]±5	195[b]±4	197[b]±5	192[b]±5	<0.05
ADG, kg/a/day	0.03[a]±0.02	0.20[b]±0.02	0.22[b]±0.03	0.18[b]±0.04	<0.05
Initial REA (cm^2)	24.3[a]±0.9	23.7[a]±1.0	23.7[a]±0.9	23.9[a]±0.6	0.96
Final REA (cm^2)	27.8[a]±0.9	27.6[a]±1.0	27.3[a]±1.0	28.1[a]±0.9	0.87
Initial FT (mm)	2.37[a]±0.06	2.36[a]±0.09	2.44[a]±0.07	2.57[a]±0.09	0.21
Final FT (mm)	2.63[a]±0.06	2.77[a]±0.12	2.62[a]±0.06	2.47[a]±0.07	0.10

[1] Means within a row with different superscripts differ (P<0.05). [2] T2: 100% high moisture sorghum grain (HMS), T2) 75% HMS + 25% sunflower expeller (SE), T3) 86.7% HMS + 12.1% SE + 1.2% urea (U), T4) 97.7% HMS + 2.3% U. [3] CP: Crude Protein in the supplement offered daily at 1% of body weight (DM basis).

Table 4 - Average daily gain (ADG), feed efficiency (FE), rib eye area (REA) and fat thickness (FT) for calves grazing natural pastures and fed high moisture sorghum grain with or without different protein sources (media ± SEM)[1]

Item	Treatment [2]					Prob.
	T1	T2	T3	T4	T5	
% CP supplement[3]	-	8.9	12.5	16.1	20.8	-
% CP from urea	-	-	30	48	47	-
Initial weight, kg	143[a]±6	141[a]±4	143[a]±4	144[a]±4	142[a]±4	0.91
Final weight, kg	148[a]±3	174[b]±4	172[b]±7	195[c]±5	185[c]±5	<0.05
ADG, kg/a/day	0.05[a]±0,03	0.32[bc]±0,04	0.29[c]±0,05	0.50[d]±0,05	0.42[bd]±0,03	<0.05
Initial REA (cm^2)	24.7[a]±1.5	24.4[a]±0.9	23.9[a]±1.3	24.3[a]±1.1	24.6[a]±1.1	0.98
Final REA (cm^2)	26.6[a]±0.8	29.4[a]±0.8	27.1[a]±1.6	28.0[a]±1.3	29.1[a]±1.0	0.41
Initial FT (mm)	2.02[a]±0.15	2.15[a]±0.12	2.25[a]±0.11	2.20[a]±0.07	2.12[a]±0.12	0.74
Final FT (mm)	2.07[a]±0.15	2.23[a]±0.09	2.31[a]±0.04	2.27[a]±0.07	2.32[a]±0.05	0.38
FE[4]	-	5.2	6.4	3.6	4.2	-

[1] Means within a row with different superscripts differ (P<0.05). [2] T2: 100% high moisture sorghum grain (HMS), T3) 97.7% HMS + 2.3% slow-release urea (SRU), T4) 94.5% HMS + 5.5% SRU, T5) 76.5% HMS + 5.5% SRU + 18.0% sunflower expeller. [3] CP: Crude Protein in the supplement offered daily at 1% of body weight (DM basis). [4] Kg of supplement (DM basis) per kg of added gain above the performance of control calves

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Based on the high response to protein supplementation in exp. 1, CP concentration of HMS was increased to 15.5% in exp. 2. The main objective was to compare sunflower expeller, urea or a combination of both as protein sources. Overall ADG and final body weight increased (P<0.05) with the addition of protein compared to fed only HMS (Table 3). Protein source had no effect on animal performance. Similarly, Ludden et al. (1995) reported that neither rate nor efficiency of growth was improved when cattle was fed 12.4% CP diets that contained one of four sources of supplemental protein at 20, 30, or 40% of the dietary CP. In another study, calves fed soybean providing higher level of ruminal escape protein showed a similar ADG than those fed a urea and corn supplement (Fernandez-Rivera et al., 1989). Summarizing experiments evaluating the efficacy of urea in supplements fed to cattle on winter range, Clanton (1978) reported decreased performance with supplements containing greater than 3% urea compared to performance of cattle receiving similar energy densities but all-natural protein supplements. In exp. 2, urea represented 1.2% (T3) and 2.3% (T4) of the total supplement on a dry matter basis. Because calves evidently were nitrogen-deficient in the present study, positive responses to the addition of protein supplements would have been expected form any protein source. In addition, sorghum protein is resistant to ruminal degradation and it is likely that a large percentage of grain protein escaped ruminal degradation increasing the demand for nitrogen rapidly available in the rumen (Merchen et al., 1987; Cecava et al., 1991). Combining a protein source high in rumen undegradable protein with a highly rumen degradable protein source can improve animal performance of grazing cattle (McMurphy et al., 2010). Even though early studies showed that changing dietary protein level can affect rib eye area and fat deposition (Dartt et al., 1978; Perry et al., 1983), in our experiment both variables were not affected by treatment with an overall increase of 16% in rib eye are and 8% in fat thickness by the end of the experiment. The age of calves, the length of the feeding period and the level of supplementation may explain the absence of response.

The objective of exp. 3 was to evaluate the response in animal performance to increasing levels of protein in the supplement. Supplementation with only HMS (T2) increased final body weight and ADG by 17.5% and 6.6 times, respectively, compared with cattle in the control group (T1). This improvement in animal performance confirms the results obtained in exp. 1 showing that energy supplementation becomes a viable alternative when the primary objective is to avoid body weight loss in low-quality pastures. In such conditions overall intake of digestible energy is increased even though forage intake can be decreased by grain-based supplements (Lamb and Eadie, 1979; Chase and Hibberd, 1987; Sanson et al., 1990; Moore et al., 1999). Final weights and daily gains were greater (P<0.05) for cattle fed supplements with 16.1% (T4) and 20.8% CP (T5) compared with those fed 8.9% and 12.5% CP (T2 and T3). Performance of calves fed either 16.1% or 20.8% CP supplements did not differ possibly because energy was becoming the limiting factor at the highest CP concentration level. Additionally, supply of key limiting amino acids may not have been increased enough to elicit a response in performance when CP was raised to 20.8% (Merchen et al., 1987). Feed efficiency for cattle in treatments T4 (3.6) and T5 (4.2) was improved by 30% and 19% compared with cattle fed diets containing only high moisture sorghum grain (5.2) confirming the results obtained in exp. 1. Ultrasound variables were not affected by treatment (P>0.05) as it happened in exp. 1 although supplemented calves registered a rib eye area and fat thickness 7% and 10% greater at the end of the experiment than un-supplemented animals.

CONCLUSIONS

The addition of protein sources to increase the high moisture sorghum grain CP concentration from 8 to 16% significantly increased the performance of calves grazing low quality pastures and daily supplemented at 1% of body weight. The utilization of sunflower expeller appears to offer no improvement in performance compared with urea at such levels. As practical recommendation the threshold of 16% CP in the sorghum grain should be reached using the available protein source most economical to accelerate the growth period of calves in extensive conditions.

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NUTRITIVE VALUE OF SAWDUST

M.E. HOSSAIN[1]*, M.J. RAHMAN[2], K.M.F. ISLAM[2]

[1]*Department of Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh*
[2]*UG student, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh*

*E-mail: emrancvasu@yahoo.com

ABSTRACT: *The present study was undertaken to observe the chemical composition of different types of sawdust available in the urban and peri-urban areas of Chittagong, Bangladesh. Twenty different types of sawdust from different plants were collected from study areas. Chemical analyses of the samples were carried out in triplicate for moisture, dry matter (DM), metabolizable energy (ME), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and total ash in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. Results indicated that, there were no variations ($P>0.05$) in the DM, EE and TA contents of the sawdust samples. However, ME, CP, CF and NFE content differed ($P<0.01$) significantly from one sample to another. DM content varied from 91.6 to 97.4 g/100g, ME content varied from 535.9 to 1756.7 kcal/kg, CP content varied from 1.8 to 3.5 g/100g, CF content varied from 39.5 to 74.0 g/100g and NFE content varied from 12.5 to 47.1 g/100g. It could therefore, be inferred that, sawdust currently available in the local market widely varies in chemical composition.*

ORIGINAL ARTICLE

Key words: *Sawdust, Dry Matter, Metabolizable Energy, Crude Protein, Crude Fiber, Nitrogen Free Extracts, Ether Extracts, Total Ash*

INTRODUCTION

The higher price and acute scarcity of conventional feed ingredients are two major constraints to the profitable commercial dairy and poultry farming. The feed cost alone accounts 60-70% of the total production cost. Computing feed with conventional feed ingredients hardly permits profitable poultry production (Bulbul and Hossain, 1989). Therefore, attention is gradually being focused on cheaper alternative feed resources, specially, crop residues and industrial by-products to sustain livestock industry. The use of unconventional feed resources along with other strategies may reduce pressure on the demand for conventional feed ingredient and promote achievement of feed security for dairy and poultry sector.

Sawdust or wood dust is a by-product of cutting, grinding, drilling or pulverizing wood with saw or other tool. It is composed of fine particles of wood. It could also be derived from certain animals, birds and insects which live in wood, such as the woodpecker and carpenter ant. Wood residues contain 70 to 80% total carbohydrate (Keith, 1976). Millions of fibrous materials like saw dust is wasted away every year from industrial sites like sugar mills and saw mills. Sawdust has been fed satisfactorily to ruminants as a roughage substitute in all concentrate rations (Marion et al., 1959; Anthony and Cunningham, 1968; Anthony et al., 1969; Dinius et al., 1970; Slyter and Kamstra, 1974; McCartor et al., 1972; Sowande, 2002). Therefore, dairy farmers who have scarcity for forages, straw and stover, may consider feeding of hard wood saw dust and wood shavings to a limited amounts. Previous studies indicate that, the inclusion of 5-15% sawdust in maize based diets for cattle was found to maintain better rumen function irrespective of few cases for bloat and liver lesions and less ruminal perakaratosis.

Sawdust is abundant throughout the whole year in developing countries. Utilization of sawdust may reduce the cost of conventional livestock feeds since it does not compete with human being. However, the problem associated with sawdust is its higher lignin content. Recent studies show that, *in vitro* dry matter digestibility (IVDMD) of sawdust by rumen microorganisms has been improved by alkali treatment (Wilson and Pigden, 1964). *In vivo* dry matter digestibility has also been improved by alkali and acid treatment of sawdust (Mellenberger et al., 1971). Therefore, the present study was aimed to investigate the chemical composition of sawdust available as saw mill by products in the urban and peri-urban areas of Chittagong, Bangladesh.

MATERIALS AND METHODS

Study area

Most of the saw mills are located in Pahartali, Khatungongja and Nasirabad areas of Chittagong metropolitan. Therefore, these places were selected as the study area for collection of sample.

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Collection of sample

Samples were collected by using simple random sampling technique. Twenty sawdust samples of different plants were selected randomly. Apporximately 500 grams of sawdust were collected as for individual plant. Samples were wrapped up by polythene bag and preserved in the laboratory for chemical analysis.

Preparation of sample

Samples were subjected to grinder to make it homogenous powder (60 mesh). Later on, it was mixed properly and exposed to shade to cool down for sampling.

Analysis of sample

Chemical analyses of the samples were carried out in triplicate for moisture, dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and total ash in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh as per AOAC (1994).

Data analysis

Data related to chemical composition of sawdust were compiled by using Microsoft Excel 2007. Chi-square (χ^2) test was performed to analyze the data by using SPSS 16.0 (Winer et al., 1991). Statistical significance was accepted at 5% level ($P<0.05$)

RESULTS

Detailed chemical composition of sawdust collected from different species of tree has been presented in Table 1 and Table 2. Results indicated that, DM, EE and TA content did not differ significantly ($P>0.05$). Minimum, maximum and mean values for DM were 91.6, 97.4 and 94.1 respectively (Table 2). Minimum, maximum and mean values for EE were 0.6, 2.0 and 1.4 respectively. Minimum, maximum and mean values for TA were 0.3, 7.6 and 1.8 respectively. ME content differed significantly ($P<0.01$). Minimum, maximum and mean values for ME were 535.9, 1756.7 and 1208.2 kcal/kg respectively. CP content differed significantly ($P<0.01$). Minimum, maximum and mean values for CP were 1.8, 3.5 and 2.4 respectively. CF content differed significantly ($P<0.01$). Minimum, maximum and mean values for CF content were 39.5, 74.0 and 56.5 respectively. NFE content differed significantly ($P<0.01$). Minimum, maximum and mean values for NFE were 12.5, 47.1 and 32.2 respectively.

Table 1 - Chemical composition of individual sawdust (N=20)

Local name	Scientific name	Nutritive value (g/100g)						
		DM	ME*	CP	CF	NFE	EE	TA
Dewa	*Artocarpus lakoocha*	94.6	1652.9	2.8	43.5	46.0	1.0	1.3
Silkoroi	*Aibizia procera*	94.6	1005.4	1.9	65.0	25.0	2.0	0.7
Akasmoni	*Acasia auriculiformis*	92.6	939.5	1.9	65.0	23.0	2.0	0.7
Arjun	*Terminalia arluna*	96.8	1563.9	2.2	49.0	43.9	1.0	0.7
Jam	*Syzygium cumini*	97.4	960.9	2.6	66.0	25.2	1.0	2.6
Jalpai	*Elaeocarpus floribundus*	97.4	1559.0	1.8	49.0	41.9	2.0	2.6
Bael	*Aegle marmelos*	96.4	1161.9	2.2	60.0	32.6	0.6	1.0
Raintree	*Samania samun*	95.2	736.9	2.5	70.0	19.4	0.6	2.7
Mahagony	*Swietenia mahagony*	95.0	1611.7	2.5	46.0	44.6	1.2	0.7
Deshi gab	*Diospyros peregrine*	94.0	1295.4	1.9	55.0	33.8	2.0	1.3
Deshi tatul	*Tamarindus indica*	92.4	615.0	2.4	66.0	15.8	0.6	7.6
Amm	*Magnifera indica*	93.0	967.5	3.5	63.0	24.5	1.0	1.0
Jambura	*Citrus grandis*	91.6	624.9	2.4	72.0	15.2	1.0	1.0
Kadam	*Anthocephalus chinensis*	91.6	535.9	2.4	73.5	12.5	1.0	2.2
Kathal	*Artocarpus heterophyllus*	92.0	1674.3	2.1	39.5	45.1	2.0	3.3
Sagun	*Tecna grandis*	93.6	1631.5	2.2	44.0	43.7	2.0	1.7
Eucalyptus	*Eucalytus teritocornis*	94.0	1644.7	2.2	44.0	44.1	2.0	1.7
Sisso	*Swietenia sissoo*	94.0	1616.7	2.2	44.0	45.5	1.0	1.3
Shimul	*Boxbax ceiba*	93.4	608.4	2.6	74.0	14.5	1.0	1.3
Chalta	*Dillenia indica*	93.0	1756.7	2.6	41.0	47.1	2.0	0.3

[DM]Dry matter; [ME]Metabolizable energy [CP]Crude protein, [CF]Crude fibre, [NFE]Nitrogen free extract, [EE]Ether extract; [TA]Total ash *kcal/kg

Table 2 - Mean chemical composition of different types of sawdust (N=20)

Parameters	Minimum	Maximum	Mean	SD	SE	Sig.
DM (g/100g)	91.6	97.4	94.1	1.80	0.40	NS
ME (kcal/kg)	535.9	1756.7	1208.2	437.3	97.8	**
CP (g/100g)	1.8	3.5	2. 4	0.39	0.09	**
CF (g/100g)	39.5	74.0	56.5	12.1	2.71	**
NFE (g/100g)	12.5	47.1	32.2	12.7	2.85	**
EE (g/100g)	0.6	2.0	1.4	0.57	0.13	NS
TA (g/100g)	0.3	7.6	1.8	1.6	0.36	NS

[DM]Dry matter; [ME]Metabolizable energy [CP]Crude protein, [CF]Crude fibre, [NFE]Nitrogen free extract, [EE]Ether extract; [SD]Standard deviation, [SE]Standard error; [NS]($P>0.05$); **($P<0.01$)

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DISCUSSION

Saw dust is a good source of dietary fibre for cattle (Anthony and Cunningham, 1968; Anthony et al., 1969; Cody et al., 1968; El-Sabban et al., 1969; El-Sabban et al., 1971; Marion et al., 1959; McCartor et al., 1972; Slyter and Kamstra, 1974), goat (Mellenberger et al., 1971), sheep (Dinius et al., 1970; Harpster, 1980), rabbit (Bederkar et al., 1984; Omole and Onwudike, 1981; Radwan, 1994), broiler (Abdelsamie, 1983; Oke and Oke, 2007) and quail (Savory and Gentle, 1976). Like conventional ingredients, sawdust contains ME, CP, CF, NFE and TA to substantial amounts (Keith, 1976; Oke and Oke, 2007; Radwan, 1994).

Radwan (1994) conducted an experiment on different types of sawdust and reported 2.53% crude protein, 0.76% ether extract, 60.26% crude fibre, 24.53% nitrogen-free extracts and 0.80% crude ash. In another experiment, Oke and Oke (2007) obtained 0.88% crude protein, 1.47% ether extract, 67.61% crude fibre and 0.64% crude ash in Ogea sawdust. These observations are in close agreement with present study.

Requirement of fibre for normal physiological functions of cattle, buffalo, goat, sheep and rabbit are well established. However, actual need and mode of utilization of fibre for poultry is controversial (Davis and Briggs, 1947). Generally, it is assumed that, excessive dietary fiber in poultry ration reduces feed efficiency, growth and egg production. However, the presence of fiber appears beneficial under certain critical cases. It was evident that, cannibalism could have been prevented by incorporation of extra fiber in poultry diet (Sheehy, 1939; Bearse et al., 1940). These study indicates that, fiber materials are not merely a source of dietary fibre, rather, in true sense, they contain effective extra nutrients essential for normal gut functioning.

Davis and Briggs (1947) used a purified source of cellulose and added to a complete diet. Results indicated that, addition of cellulose up to 15%, significantly improved growth rate. However, the exact reason for the increased growth obtained by feeding cellulose was not clear. It was assumed that hydrolysis of cellulose in the digestive tract may have contributed to a marginal extents as a growth stimulant other than simply a source of glucose derived from breakdown of cellulose (Davis and Briggs, 1947). Enzymes and other metabolites of microbiological origin might also be responsible. In fact, a wide range of microorganisms reserve the capacity to metabolize cellulose inside the gut (Baker, 1942; Hungate, 1944) and the decomposition products derived from cellulose breakdown may act as growth stimulant.

In another study, rations containing screened sawdust did not physically injure the gastrointestinal lining nor exhibit any toxic effects. Twenty-five percent sawdust was found to be the most desirable level for roughage substitution; higher levels occasionally induced impaction of digesta. Voluntary regulation of feed intake at a level comparable with Morrison's recommendation for feeding beef calves was accomplished with feeds containing 35% sawdust (Cody et al., 1968).

Addition of sawdust up to 15% to the rabbit diets had no detrimental effect on growth (Radwan, 1994). Similarly, incorporation of sawdust up to 15% did not affect feed intake. However, as the level exceeded, intake decreased gradually due to poor palatability of the diet. Similar results were obtained by other investigators (Hoover and Heitmann, 1972). In another study, addition of sawdust up to 8 g/100 did not exhibit any lethal effect (Oke and Oke, 2007).

Despite many advantages, Sibbald et al. (1960) reported a significant decrease in apparent digestible nitrogen due to incorporation of increased dietary fibre. The abrasive nature of fibre and greater volume of digesta could have caused an increase in metabolic nitrogen excretion (Hegde et al., 1978). The change in protein utilisation as a result of dietary fibre treatments may have caused changes in carcass composition. An increase in abdominal fat pad thickness associated with high fibre diets of equal energy content was found in laboratory trial.

Birds usually attempt to satisfy energy demand from voluntary intake. Therefore, increased feed consumption is usually associated with increased dietary fibre (Sibbald et al., 1960). Dietary fibre adversely affects growth rate and food conversion of birds (Abdelsamie, 1983). Similarly, high dietary fibre derived from sawdust resulted increased relative length and weight of intestine and also length of caeca (Abdelsamie, 1983; Savory and Gentle, 1976). However, this is not clear, whether fibre naturally available in foodstuffs would exert similar effects while they are in sawdust. In another study, equal concentrations of cellulose and sawdust had markedly different effects on gut morphology (Savory and Gentle, 1976).

CONCLUSION

Sawdust is a vital source of fibre for livestock. A wide range of in vivo and in vitro studies speculate that, livestock can utilize fibers available in sawdust. Additionally, it contains crude protein and ether extracts which may be used for poultry and livestock as well. Present study reveals that the quality of sawdust may vary from species to species. Therefore, it could be suggested that, sawdust should be incorporated with conventional feedstuffs at an optimal margin after laboratory analysis. However, it needs to explore more intensive studies in future to investigate sustainable methods for inclusion of this useful fibre in livestock diets.

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STRAIN EFFECT ON SOME PRODUCTIVE AND REPRODUCTIVE PERFORMANCE TRAITS OF LOCAL IMPROVED EGYPTIAN AND CANADIAN CHICKENS

A.E. TAHA[1]*, F.A. ABD EL-GHANY[2], M.M. SHARAF[1]

[1]Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Behira, Rashid, 22758 Edfina, Egypt
[2]Animal Production Research Institute, ARC, Ministry of Agriculture, Egypt

*Email: ayman_soma2007@yahoo.com

ABSTRACT: *This experiment was conducted to evaluate the effect of strain on some productive as well as some reproductive traits of local improved dual purpose three Canadian strains (Shaver A, B and C) and two Egyptian chicken strains (Salam and Mandarah). Results revealed that strain effect was evident for shaver C strain for (body weight at sexual maturity, body weight at 90 days of egg production, 42 and 65 weeks of age), also strain effect was evident for shaver C strain for feed consumption (at sexual maturity, 90 days of egg production, 42 weeks and 65 weeks of age) and (egg weight at 90 days of egg production, 42 and 65 weeks of age). While strain effect for fertility, hatchability and scientific hatchability, age at sexual maturity, Egg number at first 90 days of egg production and egg number at 42 and 65 weeks of age were recorded for Egyptian chickens. Moreover, negative correlation estimates were observed between age at sexual maturity and egg number at different periods as well as positive correlation between body weight at 8 weeks of age and most of productive traits that of high great benefits to select for economic traits in chickens at earlier age.*

ORIGINAL ARTICLE

Key words: *Strain, Egg Parameters, Egypt, Fertility, Hatchability, Correlation*

INTRODUCTION

In a developing country like Egypt, poultry production is of great importance as a primary supplier of eggs and meat and as a source of income. So, the knowledge of performance of economic traits in chicken is important for the formulation of breeding plans for further improvement in production traits. Growth and production traits of a bird indicate its genetic constitution and adaptation with respect to the specific environment (Ahmed and Singh, 2007).

Local developed stains in Egypt varied according to purpose of production; from these strains is Mandarah chickens that resulting from crossing between Alexandria male (four-way cross of Plymouth, RIR, WL and Fayoumi) and Dokki-4 female. While Salam strain is across between Nicolas male and Maamourah females for four successive generations and they are considered as dual purpose for egg and meat production.

It was found that body weights, age at sexual maturity, egg weights and egg production were significantly varied in four chicken varieties (Niranjan et al., 2008). Moreover, Sola-Ojo and Ayorinde (2011) reported that line and strain effect were evident for fertility, hatchability, body weight, total egg number, hen day egg production, body weight at first egg, and total egg number.

A number of researches have been done earlier on the relationship between body weights, age at sexual maturity, egg weight and egg production in the domestic chickens (Omeje and Nwosu, 1984; Ayorinde et al., 1988; Oni et al., 1991; Adenowo et al., 1995; Chineke, 2001; Udeh, 2010). Also, genetic and phenotypic correlations between growth and production performance of chickens were studied by many authors (Siegel and Dunnington, 1985, Nwagu et al., 2007 and El-Dlebshany 2008).

The objectives of this study were to assess the differences between local developed Egyptian and Canadian shaver chicken strains for reproductive and productive traits as well as estimation of correlation between studied parameters.

MATERIAL AND METHODS

A total number of 1951 one day old chicks obtained from three Canadian dual purpose strains received from Shaver poultry breeders and two Egyptian strains (Salam and Mandarah).

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Chicks individually weighted, sexed, wing banded and Mark's vaccinated with spectam® at one day old, then randomly distributed and put 25 females/ pen and 24 males/ pen from each strain. Chicks were floor brooded for the first five weeks of age in a clean well ventilated room, previously fumigated with formalin and potassium permanganate with ratio (2:1). The room was provided with heaters to adjust the environmental temperature according to age of the chicks, starting with 35 ^{o}C at one day old and decreased 3^{o}C weekly until the end of brooding period then adjusted at 21 ^{o}C in the growing and laying periods.

Light was provided 24 hours at the first day then decreased to 21 hours daily till the fourth week of age then reduced to 10 hours of light and 14 hours of darkness during the growing period. At the 18th weeks of age the lighting period increased gradually to 14 hours with 10 hours darkness daily. During laying period the lighting was 16 hours with darkness 8 hours daily (Chao and Lee, 2001).

During laying period males and females were subjected to optimum environments as possible to keep their high performance in cage system. Cocks were trained for semen collection (twice per week) before practicing artificial insemination by three weeks. Artificial insemination (AI) was practiced twice per week for the first week then one time per week. Hens were artificially inseminated with 0.1 ml of the fresh diluted semen (diluted with saline 0.9% by the ratio of 1:1) from its assigned cock. Semen collection was done using massage technique described by Lake and Stewart (1978) and Mostafa (1989).

Vaccination program

The program of vaccination was done as shown in Table 1:

Table 1 - vaccination program for birds

Vaccine type	Time of vaccination
Spectam 0.5 ml S/C	1st day
Hitchener B1+ Infectious Bronchitis(IB)	7th day
Gumboro (live)	13th day
Lasota	15th day
Gumboro	23rd day
Lasota +IB	30th day
Gumboro	35th day
Lasota	every 2 weeks
Infectious Bronchitis (IB)	every month

Feeding of birds

Females fed with starter ration (19% CP and 3050 K-cal/kg) ad libitum from zero to 5 weeks of age and then grower ration (14% CP- / and 3100 K-cal/kg from 6-12 weeks). Males fed with broiler starter ration (22% CP and 3150 k-cal/kg) from 0-5weeks of age, then roaster grower (20% CP and 3200 k-cal/kg) from 6- 10 weeks of age, and roaster with finisher (18% CP and 3250 K-cal/kg) from 10-12 weeks of age, finally breeder ration till the end of experiment (16% CP and 3000 k-cal /kg).

Studied traits

1- Body weight: (weight at sexual maturity, weight at first 90 days of egg production, and 42 and 65 weeks of age).

2- Age at sexual maturity: age at the first egg.

3- Fertility percentage: ((No. of fertile eggs/ Total number of eggs set)*100).

4- Hatchability percentages: Scientific hatchability percentage (No. of hatched eggs / Total number of fertile eggs)*100.

Commercial hatchability percentage (No. of hatched eggs / Total number of eggs set)*100

5- Feed consumption: was calculated at sexual maturity, first 90 days of egg production, 42 weeks of age and 65 weeks of age).

6- Feed conversion: was calculated at first 90 days of egg production, 42 weeks of age and 65 weeks of age).

7- Egg parameters: Egg Number (at first 90 days of egg production, 42 weeks of age and 65 weeks of age); Egg Weight (at first 90 days of egg production, 42 weeks of age and 65 weeks of age); Egg Mass (at first 90 days of egg production, 42 weeks of age and 65 weeks of age)

8- Estimation of correlations.

Statistical analysis:

Spearman's rank correlations were computed using SAS procedure Guide, 2004 (SAS, 2004).

The analysis of variance (GLM) for the obtained data was performed using Statistical Analysis System (SAS, 2004) software to assess significant differences according to the following model.

$X_{ijl} = \mu + G_i + e_{ijk}$

Where:

Xijk = the X th observation of the strain, μ = overall mean, Gi = effect of strain (i = Shaver A, B, C, Salam and Mandarah) and eijk= random error.

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RESULTS AND DISCUSSION

Strain effect on fertility, scientific and commercial hatchability

Fitness traits are presented in (Table 2 and Figure 1). It was observed that there were higher non-significant percentages for fertility of local Egyptian strains (Mandarah and Salam) over Canadian shaver strains C and B (93.54 and 92.14% versus 91.10 and 84.15%; respectively), while the lowest fertility percentage was recorded for Shaver A strain 68.32 %. The same trend of fertility was recorded for scientific hatchability where Mandarah and Salam strains recorded higher percentages than Shaver C, B and A (95.32, 93.12% versus 88.59, 83.88 and 82.35 %; respectively), Moreover, commercial hatchability percentages were higher for Mandarah and Salam strains than those of Shaver C, B and A (89.47, 87.30% versus 80.46, 70.95 and 56.18 %; respectively). These results confirmed by those obtained by (Sola-Ojo and Ayorinde, 2011) who found significant ($P<0.05$) effect of genotype on fertility and hatchability of eggs. Higher fertility and hatchability percentages for local breeds over exotic ones also were reported by (Horst, 1991 and Dessie and Ogle, 2001). Moreover, breed differences for fertility percentage were reported by (Kamble et al., 1996) while breed differences for hatchability percentage were recorded by (Alaba, 1990; Atteh, 1990 and Fayeye et al., 2005).

From the above results it was clear that local Egyptian chicken strains (Salam and Mandarah had superiority for fitness traits than Canadian Shaver strain A, B and C. This superiority may be due to adaptation to the Egyptian environmental conditions.

Table 2 - Least square means ± standard errors of the effect of different strains on Fertility, Scientific hatchability and Commercial hatchability

Parameter / Strain	Fertility	Scientific hatchability	Commercial hatchability
Shaver A	68.32±3.75 ^{b}	82.35±2.45 ^{b}	56.18±5.11 ^{c}
Shaver B	84.15±3.92 ^{a}	83.88±3.36 ^{b}	70.95±5.47 ^{bc}
Shaver C	91.10±3.01 ^{a}	88.59±3.92 ^{ab}	80.46±3.88 ^{ab}
Salam	92.14±2.58 ^{a}	93.12±3.44 ^{ab}	87.30±5.18 ^{ab}
Mandarah	93.54±2.41 ^{a}	95.32±2.94 ^{a}	89.47±5.18 ^{a}

a, b and c = means on the same column (for the average of strains) significantly ($p \leq 0.01$).

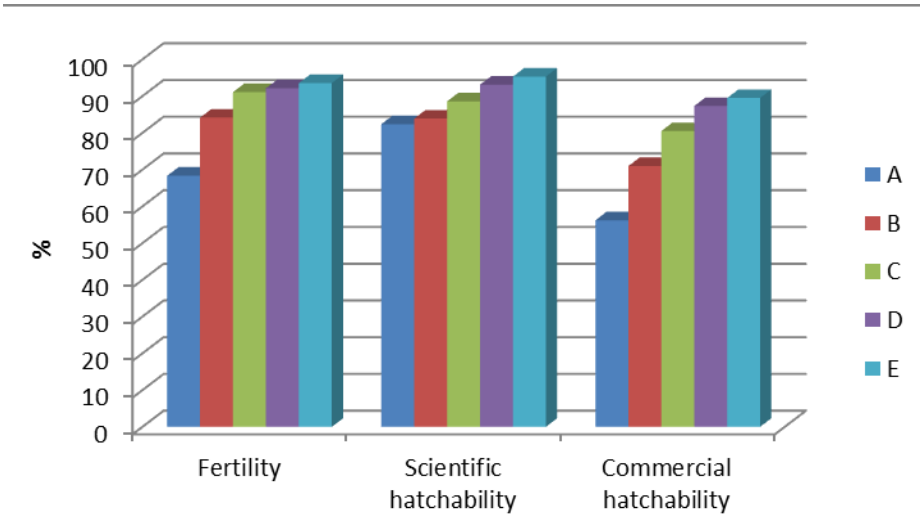

Figure 1 - Fertility, Scientific and commercial hatchability percentages among three Canadian and two Egyptian local strains. A, B, C, D and E =(Shaver A, Shaver B, Shaver C, Salam and Mandarah)

Strain effect on body weight

Results of body weight for different local Egyptian and Canadian chicken strains at different periods are presented in (Table, 3).

Body weight at sexual maturity

It was observed that shaver C strain reached sexual maturity with the heaviest weight (2661.34 g) followed by shaver A (1873.38 g) while the lowest body weight at sexual maturity was recorded for Shaver B strain (1615.63 g). Strain and line effects for body weight at sexual maturity were also recorded by (Udeh, 2010 and Sola-Ojo and Ayorinde, 2011 and El-labban et al., 2011).

Body weight at 90 days of egg production, 42 and 65 weeks of age

Shaver C strain recoded superiority in body weight at 90 days egg production over other studied strains (2832.66 g) followed by Shaver A strain (2100.51 g), but the lowest body weight recorded for Mandarah strain

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(1960.70 g). The same trend was recorded for body weight at 42 weeks of age where the highest body weight was recorded for Shaver C strain (3157.21 g) followed by Salam strain (2172.21 g), while the lowest body weight recorded for Mandarah strain (2100.90 g). These results confirmed by those obtained by (Niranjan et al., 2008 and Yahaya et al., 2009) who found strain differences for body weight at 40 weeks of age. In addition Shaver C strain also recorded the highest body weight at 65 weeks at age (3388.76 g) followed by Shaver A (2309.88 g). Similar results obtained by (Niranjan et al., 2008) who found significant differences between different layer strains at 64 weeks of age. On the other hand, Mandarah strain had the same trend of body weight at 90 days of egg production and 42 weeks of age and recorded the lowest body weight (2127.60 g). Strain effect for body weight were also recorded by (Ojedapo et al., 2008 and Singh et al., 2009) who found that there were line and strain effect for body weight at 30, 40 and 50 weeks of age for four strains of laying hens.

Strain effect on age at sexual maturity

Age at sexual maturity for different local Egyptian and Canadian chicken strains are summarized in (Table, 3 and Figure 2). Egyptian Mandarah strain reached sexual maturity earlier than other strains (151.60 days) followed by Salam strain (163.66 days), while Canadian Shaver B strain reached sexual maturity at older age (181.87 days). It was noticed that Egyptian strains reached sexual maturity at earlier age than Canadian Shaver strains. Differences in age at sexual maturity between different lines of poultry were also recorded by (Udeh, 2007; Niranjan et al., 2008; Yahaya et al., 2009; Udeh, 2010; El-labban et al., 2011; Udeh and Omeje, 2011), but disagree with AL-Nasser et al., 2008 who found that there were no differences for age at sexual maturity for Lohmann LSL-Classic white and brown strains.

Strain effect on feed consumption

Feed consumption at different periods in local Egyptian and Canadian chicken strains are listed in (table, 3). Higher significant differences for feed consumption at sexual maturity for Shaver C strain (146.59 g), followed by Mandarah strain (127.00 g), while the lowest feed consumption recorded for Shaver A (103.20 g). The same trend for feed consumption at 90 days of egg production was recorded for Shaver C (140.36 g) followed by Shaver A (133.47 g), on the other hand Mandarah strain recorded the lowest feed consumption (128.48 g). Shaver C strain also, recorded the highest significant for feed consumption at 42 weeks and 65 weeks of age (142.64 and 145.12 g; respectively) while Salam strain recorded the lowest feed consumption at the same periods (130.77 and 131.24 g; respectively). The results agreed with those obtained by Lacin et al., 2008 who found Strain effect for feed consumption among different layer strains.

Table 3 - Least square means ± standard errors of the effect of different strains on body weight, age at sexual maturity and feed consumption.

Parameter*	Strains					Average
	Shaver A	Shaver B	Shaver C	Salam	Mandarah	
Body weight						
1	1873.38±19.10^{b}	1615.63±22.45^{d}	2661.34±32.37^{a}	1728.73±27.35^{c}	1649.60±18.49 ^{d}	1903.76±20.69
2	2100.51±17.67^{b}	1977.97±16.64^{c}	2892.66±23.25^{a}	1998.63±23.36^{c}	1960.70±13.97 ^{c}	2184.67±18.28
3	21.59.05±13.17^{b}	2119.79±15.18^{bc}	3157.21±26.26^{a}	2172.21±22.69^{b}	2100.90±15.80 ^{c}	2340.51±20.39
4	2309.88±27.34^{b}	2229.39±24.30^{b}	3388.76±40.25^{a}	2279.57±26.82^{b}	2127.10± 22.71^{c}	2464.96±24.69
Age at SM	160.14±0.54 ^{d}	181.87±0.33 ^{a}	166.73±0.24 ^{b}	163.66±0.62 ^{c}	151.60 ± 0.54 ^{e}	164.80±0.50
FC1	103.20±5.10 ^{d}	120.87±0.65 ^{c}	146.59±0.27 ^{a}	127.00±0.16 ^{bc}	127.73±0.13 ^{b}	125.11±1.19
FC2	133.47±0.18 ^{b}	131.01±0.16 ^{c}	140.36±0.15 ^{a}	128.48±0.08 ^{e}	129.50±0.05 ^{d}	132.55±0.20
FC3	135.65±0.12 ^{b}	133.88±0.14 ^{c}	142.64±0.13 ^{a}	130.98±0.10 ^{d}	130.77±0.08 ^{d}	134.77±0.20
FC4	137.81±0.06 ^{b}	135.41±0.12 ^{c}	145.12±0.15 ^{a}	131.49±0.11 ^{d}	131.24±0.08 ^{d}	136.19±0.23

$^{a, b, c, d \text{ and } e}$ means on the same raw (for the average of strains) significantly (P≤0.01). Body weight 1, 2, 3 and 4, Age at SM, FC1, FC2, FC3 and FC4= body weight at age at sexual maturity, body weight at 90 days of production, body weight at 42 weeks of age, body weight at 65 weeks of age, age at sexual maturity, feed consumption at sexual maturity, feed consumption at 90 days of production, feed consumption at 42 weeks of age and feed consumption at 65 weeks of age

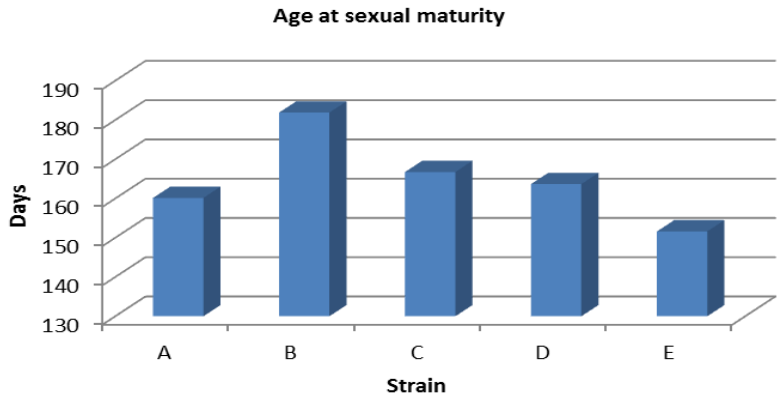

Figure 2 - Age at sexual maturity(days) between three Canadian and two Egyptian local strains A, B, C, D and E = (Shaver A, Shaver B, Shaver C, Salam and Mandarah)

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Strain effect on egg parameters

Egg number, weight and egg mass for different periods in local Egyptian and Canadian Chicken strains are presented in (Table, 4).

Table 4 - Least square means ± standard errors of the effect of different strains on egg production (egg number, egg weight and egg mass) and feed conversion.

Parameter	Strains					Average
	Shaver A	Shaver B	Shaver C	Salam	Mandarah	
EN1	46.42±0.76 c	35.83±0.40 e	44.48±0.56 d	61.75±0.30 d	65.92±0.54 a	50.88±0.56
EN2	101.71±0.49 c	71.83±0.72 d	100.36±0.34 c	118.57±0.16 b	123.14±0.55 a	102.97±0.85
EN3	179.65±0.72 c	130.63±1.21 e	160.51±0.48 d	191.01±0.49 b	199.94±0.68 a	172.25±1.17
EW1	63.24±0.24 c	64.16±0.14 b	66.83±0.19 a	51.28±0.08 d	50.46±0.03 e	59.18±0.32
EW2	68.64±0.14 b	62.72±0.13 c	71.33±0.25 a	55.62±0.06 d	53.94±0.08 e	62.42±0.31
EW3	67.05±0.07 b	64.45±0.22 c	70.45±0.25 a	56.16±0.03 d	55.14±0.08 e	62.73±0.28
EM1	2937.36±49.55c	2294.67±21.88 d	2967.88±34.07 c	3165.30±12.74b	3327.40±28.19 a	2937.34±21.39
EM2	6977.72±24.42b	4482.15±50.93 d	7154.11±22.31 a	6595.60 ± 9.76 c	6645.17±36.49 c	6362.17±36.49
EM3	12046.56±50.70a	8439.30±102.35e	11391.59±60.05 b	10728.14±30.26d	11023.69±32.70c	10715.58±62.15
F.conv.1	4.20±0.07 b	5.18±0.04 a	4.31±0.05 b	3.65±0.01 c	3.52±0.03 d	4.17±0.03
F.conv.2	3.50±0.01 d	5.43±0.05 a	3.59±0.01 b	3.57±0.04 c	3.55±0.02 c	3.93±0.04
F.conv.3	4.01±0.02 c	5.69±0.07 a	4.47±0.02 ab	4.29±0.01 b	4.16±0.01 bc	4.53±0.03

a, b, c, d and e means on the same raw (for the average of strains) significantly ($p \leq 0.01$). EN1, EW1, EM1, EN2, EW2, EM2, EN3, EW3 and EM3= Egg number at first 90 days of production, average egg weight at first 90 days of production, Egg mass at first 90 days of production, Egg number at 42 weeks of age, average egg weight at 42 weeks of age, Egg mass at 42 weeks of age, Egg number at 65 weeks of age, average egg weight at 65 weeks of age and Egg mass at 65 weeks of production; respectively

Egg number

Egg number at first 90 days of production (Table, 4 and Figure 3) revealed that Salam strain recorded the highest significant values for egg production followed by Mandarah strain (65.92 and 61.75), while the lowest egg number recorded for Shaver B strain (35.83). Also, egg number at 42 weeks of age was of highest significant for Salam strain followed by Mandarah strain (123.14 and 118.57; respectively), while Shaver B recorded the lowest egg number (71.83). Significant strain differences for egg number at first 90 days of age were also recorded by (El-labban et al., 2011).

Salam strain continues recoding the highest significant egg number at 65 weeks of age followed also by Mandarah strain (199.94 and 191.01; respectively). On the other hand the worst egg number recorded for Shaver B strain (130.63). It was clear that there were superiority for number at different periods of production for Egyptian Local strains (Salam and Mandarah) over Canadian shaver Strains. Strain differences for egg production at different ages of laying hens where reported by (Udeh, 2007; Lacin et al., 2008; Niranjan et al., 2008; Yahaya et al., 2009; Sola-Ojo and Ayorinde, 2011; Udeh and Omeje, 2011).

Egg weight

It was noticed that Shaver C recorded the highest significant differences for egg weight (Table, 4 and Figure 4) at 90 days of egg production, 42 and 65 weeks of age (66.83, 71.33 and 70.45 g; respectively), while the lowest egg weights for the periods were recorded for Salam strain (50.46, 53.94 and 55.14 g; respectively). Results agreed with those obtained by Udeh, 2007 who reported that the comparative performance between the two strains of chicken showed significant differences in weight of first egg, egg weight at 30 and 40 weeks. Also strain differences for egg weight were recorded by Lacin et al., 2008; Niranjan et al., 2008; Yahaya et al., 2009; Udeh and Omeje, 2011). It was clear that egg weights were negatively correlated with egg number as observed in Salam strain.

Egg mass

Salam strain was of highest significant values for egg mass (Table, 4 and Figure 5) at 90 days of egg production (3327.40 g), while shaver B recorded the lowest egg mass (2294.67 g), but egg mass at 42 weeks of age was of highest significant values for Shaver C (7154.11 g) and the lowest egg mass also recorded for Shaver B (4482.15 g). On the other hand egg mass at 65 weeks of age was significant for Shaver A (12046.56 g) and Shaver B was still of the lowest egg mass (8439.30 g). The results in agreement with those obtained by (El-labban et al., 2011) who found strain differences for egg mass at first 90-days, egg mass for 210-days, egg mass for first ten eggs, egg mass for one week per month and egg mass for two days per week. Strain effect for egg mass also recorded by (Udeh, 2007).

Strain effect on feed conversion

From the data presented in (Table, 4) Salam and Mandarah strains represented the best feed conversion rate at first 90 days of production 3.52 and 3.65 kg, while Shaver A strain recorded the best feed conversion at 42 weeks of age (3.50 kg) followed by Salam and Mandarah strains (3.55 and 3.57 Kg), more over the same trend was recorded for feed conversion at 65 weeks of age; Shaver A strain showed the highest feed conversion ratio (4.01kg) followed by Salam and Mandarah strains (4.16 and 4.29 Kg). From the mentioned results Egyptian Salam and Mandarah strains represented best feed conversion over Shaver B and C Strains. The same results reported by Udeh, 2007 who found significant strain effect for feed conversion into eggs between two strains of

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brown Nick and Black Olympia layer type chickens. Strain effect for feed conversion in different layer strains was also recorded by Lacin et al., 2008.

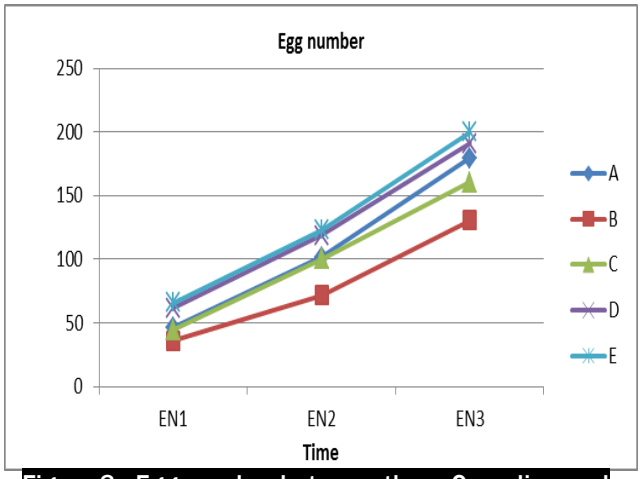

Figure 3 - Egg number between three Canadian and two Egyptian local strains

A, B, C, D and E =(Shaver A, Shaver B, Shaver C, Salam and Mandarah) EN1, EN2 and EN3=(Egg number at first 90 days of production, 42 and 65 weeks of age

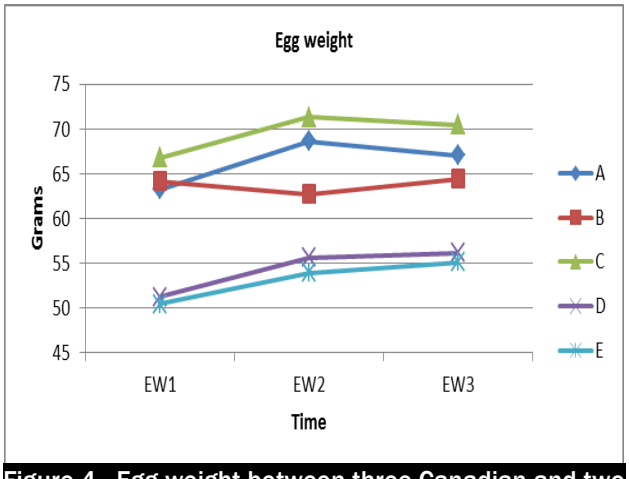

Figure 4 - Egg weight between three Canadian and two Egyptian local strains

A, B, C, D and E =(Shaver A, Shaver B, Shaver C, Salam and Mandarah) EW1, EW2 and EW3=(Egg weight at first 90 days of production, 42 and 65 weeks of age

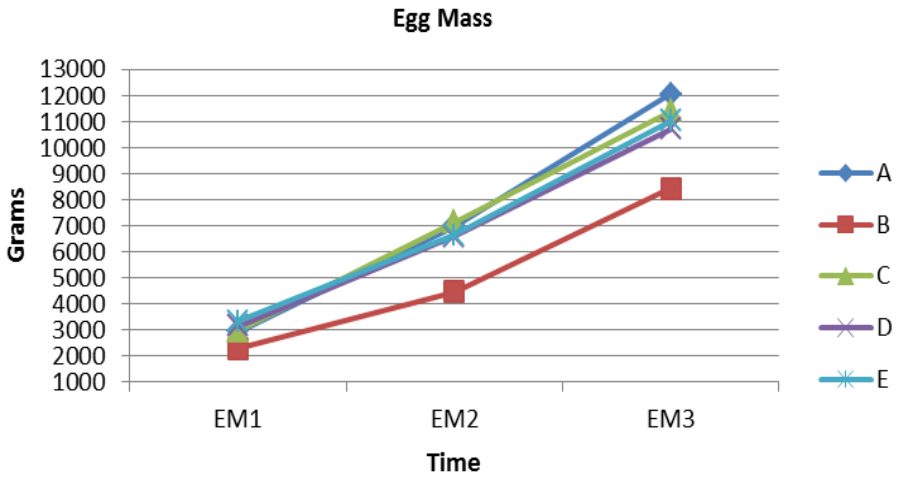

Figure 5 - Egg mass between three Canadian and two Egyptian local strains

A, B, C, D and E =(Shaver A, Shaver B, Shaver C, Salam and Mandarah) EM1, EM2 and EM3=(Egg mass at first 90 days of production, 42 and 65 weeks of age

Correlations among some productive traits

Correlation coefficients among some production traits were presented in table (Table 5). It was observed that there were highly positive correlations between body weights at 8 weeks, body weight at first 90 days, body weight at first 42 weeks of age and body weight at first 65 weeks of age. While negative correlation values were recorded between BW1, BW2, BW3 and Sexual maturity (-0.13, -0.02 and -0.05) on the other hand mild positive correlations were recorded between BW4, BW5 and Sexual Maturity (0.06 and 0.07). These results agreed with those obtained by (Udeh, 2010) who found that the genetic and phenotypic correlations of age at sexual maturity were negative with all of body weight at 4-wk, 8-wk of age,

Negative correlation estimates were observed for EW1 and EN1, EW2 and EN1, EW3 and EN1 (-0.84, -0.65 and -0.71; respectively), also EW2 and EN2, EW3 and EN2 (-0.49 and -0.56), in addition EW3 and EN3 (-0.55). These results agreed with those obtained by (Veeramani et al., (2008) and El-labban et al., 2011). But not agreed with those obtained by Nwagu et al., (2007) who reported that correlation between egg number and egg weight was small non-significant. On the other hand, Positive correlation estimates were recorded between EN1 and EM1, EN2 and EM2, EN3 and EM3 (0.89, 0.76 and 0.72; respectively).

Highly negative correlation estimates were observed between age at sexual maturity and EN1, (-0.70), EN2 (-0.87) and EN3 (-0.83). The same results were obtained by Veeramani et al. (2008) who found negative correlation between ASM and Egg production on both genetic and phenotypic scale.

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Table 5 - Correlation coefficients among some production traits

Parameter	BW1	BW2	BW3	BW4	BW5	SM	EN1	EW1	EM1	EN2	EW2	EM2	EN3	EW3	EM3	F.Con1	F.Con2	F.Con3
BW1	-	0.68**	0.69**	0.73**	0.70**	-0.13**	0.01	0.26**	0.22**	0.20**	0.43**	0.53**	0.05	0.40**	0.57**	-0.16**	- 0.41**	- 0.24**
BW2		-	0.90**	0.86**	0.78**	-0.02	-0.22**	0.50**	0.06	-0.02	0.63**	0.43**	-0.14**	0.60**	0.32**	0.04	-0.28**	-0.11*
BW3			-	0.94**	0.84**	-0.05	-0.28**	0.53**	0.00	-0.07	0.64**	0.39**	- 0.20**	0.63**	0.28**	0.10	-0.23**	-0.07
BW4				-	0.87**	0.06	-0.25**	0.50**	0.02	-0.06	0.60**	0.37**	-0.26**	0.60**	0.24**	0.08	-0.215**	-0.05
BW5					-	0.07	-0.28**	0.51**	-0.03	-0.08	0.60**	0.35**	-0.22**	0.58**	0.21**	0.12**	-0.20**	-0.02
SM						-	-0.70**	0.54**	-0.65**	-0.87**	0.33**	-0.67**	-0.83**	0.41**	-0.64**	0.70**	0.76**	0.71**
EN1							-	-0.84**	0.89**	0.86**	-0.65**	0.48**	0.85**	-0.71**	0.40**	-0.93**	-0.62**	-0.59**
EW1								-	-0.50**	-0.74**	0.89**	-0.16**	-0.73**	0.93**	-0.07	0.61**	0.36**	0.35**
EM1									-	0.77**	-0.27**	0.65**	0.76**	-0.33**	0.61**	-0.98**	-0.72**	-0.68**
EN2										-	-0.49**	0.76**	0.93**	-0.56**	0.62**	-0.82**	-0.87**	-0.77**
EW2											-	0.19**	-0.49**	0.97**	0.23**	0.39**	0.00	0.05
EM2												-	0.68**	0.09	0.88**	-0.62**	-0.98**	-0.83**
EN3													-	-0.55**	0.72**	-0.80**	-0.81**	-0.87**
EW3														-	0.19**	0.46**	0.11*	0.09
EM3															-	0.58**	-0.87**	-0.95**
F.Con1																-	0.73**	0.68**
F.Con2																	-	0.88**
F.Con3																		-

BW1, BW2, BW3, BW4, BW5, SM, EN1, EW1, EM1, EW2, EM2, EN2, EN3, EW3, EM3, F.Con1, F.Con2 and F.Con3= body weight at 8 weeks of age, body weight at sexual maturity, body weight at first 90 days, body weight at first 42 weeks of age, body weight at first 65 weeks of age, age at sexual maturity, egg number at 42 weeks, egg weight at 42 weeks, egg mass at 42 weeks, egg number at first 90 days of production, egg weight at first 90 days of production, egg mass at first 90 days of production, egg number at 65 weeks, egg weight at 65 weeks, feed conversion at first 90 days of egg production, feed conversion at 42 weeks and feed conversion at 65 weeks of age

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CONCLUSION

From the above results we can conclude that Canadian Shaver C strain recorded the best results for most productive traits, while Egyptian strains (Salam and Mandarah) recorded the best results for reproductive traits as well as egg numbers. Also, we can select for body weight at eight weeks of age for improving most of productive traits as egg number, egg weight and egg mass instead of selection in older ages of birds that will be economically more benefit.

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EVALUATION OF INDIRECT ELISA IN DIAGNOSIS OF NATURAL OVINE CYSTICERCIOSIS AND HAEMONCHOSIS

K. SULTAN[1]*, A.Y. DESOUKY[1], N.M. ELBAHY[2], M.A. ELSIEFY[1]

[1]*Department of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Menufyia University, Egypt*

*E-mail: Khsultan149@hotmail.com

ABSTRACT: *This study aimed to evaluate the sensitivity and specificity of indirect ELISA in diagnosis of natural infection of sheep with Cysticercus tenuicollis and Haemonchus contortus the most prevalent parasitic helminths in Egyptian sheep. By using non-purified crude antigens derived from the whole cyst of C .tenuicollis and adults H.contortus in the indirect ELISA assay; the results showed that both antigens sensitivity were 90%, 87.5% and the specificity were 60% and 75% respectively. These data proves the suitability of ELISA in diagnosis of such infections in living animals and the necessitation of using purified antigens rather than non-purified to increase the accuracy of the assay.*

SHORT COMUNICATION

Key words: *ELISA, Ovine, Cysticercus, Haemonchus*

INTRODUCTION

Sheep considers one of the most promising animals to achieve the aims of animal products supplies for the human being Haenlein and Abdellatif (2003). The infection with larval stage of *Taenia hydatigena (i.e. Cysticercus tenuicollis)* is considered one of the most wide spreading parasitic diseases infecting sheep all over the world causing considerable economic losses Abidi et al. (1989). In the other hand, *Haemonchus contortus* is regarded as the most important gastrointestinal nematodes infecting sheep in tropical and subtropical countries Sissay et al., (2007). In Egypt, *C. tenuicollis* and *H. contortus* infection in sheep were recorded with high prevalent Sultan et al., (2010).

For accurate identification of the digestive tract nematodes, the most wide spread method is fecal examination which includes fecal egg count and fecal larval culture; these methods requires experience, time-consuming and have doubtful results Eysker and Ploeger (2000) and moreover the ordinary diagnostic procedures for Ovine cysticercosis pre-mortem are useless El-Massry, (1988). While, the utilize of serological tests such as Enzyme Linked Immunosorbent assay (ELISA) are more sensitive and specific than the conventional methods of diagnosis of parasitic infections Ndao (2009).

This study was designed to evaluate the sensitivity and specificity of indirect ELISA in diagnosis of *C. tenuicollis* and *H. contortus* in sheep.

MATERIALS AND METHODS

During postmortem examination of sheep slaughtered in El-Mahalla El-Kubra abattoir, *C. tenuicollis and* adult *H. contortus* were collected, washed, examined and identified to species level according to available identification keys. Also, blood samples were individually collected in clean screw Falcon tubes, centrifuged at 3000 rpm/5 minutes to obtain clear sera, which were ambulated, labeled and preserved at -20 ̊C until use.

Cysticercus tenuicollis whole cyst prepared according to Elmassry (1999), while adult *Haemonchus contortus* crude antigens prepared according to Kandil (1994). The protein content of each was determined according to modified Lowry's assay (1951). Prepared antigens were ambulated and stored at -20 ºC until used. In order to figure the optimum dilutions of both serum and antigen checkerboard titration. *H.contortus* crude somatic antigen and *C. tenuicollis* whole cyst antigen) diluted at their optimal concentration, ELISA plate wells filled with 100 µl of the antigen, incubated, washed , blocked by 1% bovine serum albumin, and re-incubated, re-washed, sera diluted (in ratio 1: 100) were added, re-incubated, washed, adding of conjugate (i.e. Anti-sheep IgG whole molecule Alkaline Phosphatase, produced by Sigma® (used as instruction of the manufacturer), incubated wells, washed, Substrate (i.e. P- nitrophenyl phosphate produced by Sigma® and used as instruction of the manufacture), incubated, reaction appears with yellow coloration, stopped by addition of 1N NaoH 50 µl/ well and measured using ELISA reader (star Fax 303+, 12 well strips) at absorbance 405 nm.

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RESULTS AND DISCUSION

Sera collected from naturally infected sheep with *C.tenuicollis* (n=30) and free from infection (n= 10) were tested by indirect ELISA with whole cyst antigen concentration equals to 40 µg/ ml, the cutoff value (which calculated as double fold of mean of the negative sera) for *C.teniucollis* whole cyst antigen was 0.293 and 27 of 30 sera obtained from naturally infected sheep, this means 90% sensitivity. Four sera samples derived from apparently non-infected sheep gave positive reaction, so sensitivity was 60%.

While, *H. contortus* adult crude somatic antigen used with concentration equals to 40 µg/ ml; the cutoff value, was 0.326. Seven out of 8 derived from naturally infected sheep that were harboring *H.contortus* gave positive reaction (87.5% sensitivity), whereas 2 sera samples derived from sheep free from *H.contortus* in their abomasum gave positive reaction, so sensitivity was 75%.

Using of immunological assays as a tool for diagnosis of helminths infection seems promising tools. The results of *C.teniucollis* whole cyst antigen agreed with results of El-Massry (1999). The results of *H.contortus* adult crude somatic antigen agreed with results obtained by Handrilix (1990) and Schallig (1994). The considerable low level of sensitivity and specificity of both used antigens may be attributed to the antigens which used was crude non-purified, non-characterized antigen. Indicating that ELISA assay is rapid, easy and sensitive assay can be used in diagnosis of infections especially helminths infections, but must consider that its results depends mainly on the type of antigen which used and sera which used as a control positive and/or negative, in few words to obtain the best results, should use specific, purified antigen with positive control hyper-immune sera prepared in suitable lab animal and the negative control sera preferred to be sera of another host rather than animal species in the research.

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GROWTH OF POULTRY CHICKS FED ON FORMULATED FEED CONTAINING SILK WORM PUPAE MEAL AS PROTEIN SUPPLEMENT AND COMMERCIAL DIET

A. DUTTA, S. DUTTA, S. KUMARI

[1]*Department of Zoology, Ranchi University, Ranchi-834008, India*
[2]*Department of Zoology, Ranchi Women's College, Ranchi-834001, India*

Email: sweety 25j@gmail.com

ORIGINAL ARTICLE

ABSTRACT: *Waste silkworm pupae (SWP) generate vast resources of nutrients for livestock and poultry. In the present investigation, three days old chicks of RIR strain were allocated to five dietary treatments of silk worm pupae meal. The energy budget was prepared from calculated proximate analysis and growth performance of broiler chicks fed with different percentages of silk worm pupae. The result showed that the silkworm powder meal (SWPM) is the cheapest and has potential to replace the costly and contaminated fish meal, as the protein source, used in poultry industry.*

Key words: *Poultry; Fish Meal; Silkworm Pupae Meal; Proximate Analysis; Growth Performance; Energy Budget*

INTRODUCTION

Broiler industry provides not only a good source of protein but also employment. Poultry meat contributes about 37% to the total animal protein consumption in India (Ahmed and Islam, 1990) and so broiler industry is gaining importance due to increasing demand of animal protein. But broiler producers are facing difficulty on account of availability and prices of feed ingredients. Feed cost account 65-70% of total poultry rearing cost (Bhuiyan, 1989). Compared to the other nutrient sources, animal protein is the most costly ingredient for formulation of poultry diets, account 15% of feed cost (Banerjee, 1992; Singh, 1990). Fish meal (FM) is the only conventional animal protein source for poultry and as a result the cost of fish meal (FM) is very high and its inclusion in diet hardly permits profitable poultry farming. In rural India, FM supply is not only very uncertain but also usually contaminated, may even contain lethal pesticides deleterious to poultry industry (Khatun et al., 2003).

Little work has been done in India to replace the traditional animal protein supplements in animal feed with by-products of agro-industrial origin (Wijayasinghe and Rajaguru, 1977). In silk industry silk worm pupae (SWP) are discarded after reeling of silk thread, which contains a high percentage of protein that can and have been experimentally used as a animal feeds for chicken, pigs, rabbits and cattle and also for freshwater fish (Das and Sutradhar, 1971). Silk worm pupae of *Antheraea mylitta*, Drury, a waste product of silk industry, is not only rich in protein (Bhuiyan, et al.,1998) but also is also an important source of Nitrogen, Calcium, Phosphorus, Crude Fibre, Lysine, Metheonine, etc. (Habib and Hasan, 1995).

Keeping the above facts in mind the present investigation was undertaken to see whether this unconventional but important protein and energy source for poultry can be utilized in replacement of FM for optimum performance of broiler chicks. The present study deals with evaluating silkworm pupae (SWP) as an economic substitute of protein concentrate (PC).

MATERIAL AND METHODS

Unused silkworm pupae were collected from Central Tasar Research and Training Institute (CTR&TI), Ranchi, Jharkhand. The pupae were sun dried, powdered and used as silkworm pupae meal (SWPM). Three days old chicks of RIR strain were allocated to five dietary treatment groups: Group-1 (Gr.1) (100% FM + 0% SWP), Group-2 (Gr.2) (75% FM + 25% SWP). Group-3 (Gr.3) (50% FM +50% SWP), Group-4 (Gr.4) (25% FM + 75 % SWP) and Group-5 (Gr. 5) (0% FM + 100% SWP) in three replications, each were having 10 birds. Replication-wise body weight gain (g/day), feed intake (g/ day /bird) and growth performance were calculated. The energy budget was prepared according to Samuel et al. (2004). The data were statistically analyzed.

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RESULTS

Table 1 shows the proximate composition of feed used in the present study. The feed were so prepared that only the fish meal (FM) was replaced by Silkworm pupae, at varying percentage in different groups. Group 3 has highest ash content (8.254 in 7-8 week) while phosphorus content is slightly higher in Gr. 3 (0.456 in 7-8 week), than other experimental groups. The protein content of left over feed is highest in Gr. 4 after 8 weeks. While the amount of crude fiber and lipid was maximum in Gr. 4 after 6 weeks. Other parameters are shown in Table 1 for all the groups during the entire treatment period.

Table 1 - Proximate analysis of poultry feed on DM basis feed offered

	0 – 6 Week					7 – 8 Week				
Particular	**Gr 1**	**Gr 2**	**Gr 3**	**Gr 4**	**Gr 5**	**Gr 1**	**Gr 2**	**Gr 3**	**Gr 4**	**Gr 5**
Crude protein	21.88	20.92	20.84	20.89	20.88	23.254	23.45	23.456	23.595	23.53
Crude fiber	4.102	4.201	4.105	4.406	4.321	4.202	4.314	4.215	4.312	4.313
Lipid	2.245	2.631	2.734	2.634	2.678	2.345	2.249	2.543	2.615	2.532
Total Ash	6.885	7.145	6.956	6.856	6.989	6.956	7.005	8.254	7.105	7.981
Calcium	1.125	1.25	1.254	1.321	1.312	1.325	1.254	1.095	1.129	1.112
Phosphorus	0.456	0.321	0.356	0.41	.359	0.51	0.432	0.456	0.412	0.432

When a nutritional study was carried out to know the feasibility of formulated feed containing different % of Silkworm pupae (SWP) by replacing fish meal (FM) in identical percentage on the growth and conversion efficiency of poultry chick, it was found that the best relative growth was observed in Gr. 1 followed by Gr. 3, while Gr. 5 performed poorly (Table2). The gain in the body weight by the chicks in different groups is presented in table 3 which showed that the increase in the weight was maximum in Gr. 3 as compared to other groups.

Table 2 - Growth performance of broiler chicks on diets containing different levels of silkworm pupae powder

Variable	**Dietary Groups**				
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**
Live Weight at Start of Expt (g/bird)	45.16±0.24	43.4±0.62	48.02±0.35	45.4±0.62	47.6±0.5
Live Weight at end of Expt (g/bird)	1573.8±1.81	1543.52±0.29	1576.4±0.62	1532.22±0.44	1462.02±0.29
Weight gain (g/day)	25.06±0.24	24.59±0.01	25.05±0.18	24.37±0.17	23.19±0.11
Feed consumption (g/day/bird)	16.58±0.20	16.2±0.15	16.3±0.16	16.07±0.14	15.58±0.15

Gr-1 (100% FM + 0% SWP), Gr-2 (75% FM + 25% SWP), Gr-3 (50% FM +50% SWP), Gr-4 (25% FM + 75 % SWP) and Gr-5 (0% FM + 100% SWP); FM (fish meal); SWP (silk worm pupae meal)

Energy budget of chick fed on different level of SWP are presented in Table 4. Comparatively higher production was observed in Gr. 1, followed by Gr. 3. Gr. 3 also showed highest assimilation ratio, followed by group 1. Higher gross growth efficiency K_1 (142.63%) and net growth efficiency K_2 (140.47%) was observed in feeding gr.1 (fed exclusively with commercial feed with 100% F.M.) and lowest K_1 & K_2 in Gr. 5. Gr.3 was next to Gr. 1 in K_1 and K_2 value as shown in Table 4. Hence Gr. 3 with cheaper SWP may be considered to be suitable alternative to commercial feed containing costlier FM.

Keeping the feed cost low and at the same time providing a balanced diet to poultry has been the main concern of both the poultry production and feed manufacturer. Economics of feed cost without impairing poultry production can be achieved by formulating low cost diets by appropriate selection of feed ingredients. Keeping this in mind and gradually replacing the costly fish meal by cheaper silkworm pupae meal, it is possible to reduce the overall cost of chicken production.

The economics of broiler production under the five regimes of feeding in the present investigation has been calculated based on the cost per kg live weight gain as shown in Table 5 which is dependent on the cost of ingredients used for their feeding and the feed efficiency in various feeding groups.

Cost of the feed ingredients has been detailed in Table 5 and the same have been calculated on the basis of cost price at the local market of Upper Bazar Ranchi. The perusal of data incorporated in the table revealed that the cost of total feed offered was lowest in where SWP replaced 100% protein of fish meal. The cost of feed offered was highest for Gr.1.

This shows that with a linear increase of incorporation of SWP in the poultry feed there is corresponding decrease in cost per unit of feed. However the cost per kg live weight gain shown in Table 5 was lowest in Gr.3 followed by Gr. 4, Gr. 2 and lastly Gr. 1 respectively. The cost per kg live weight gain was comparatively higher in Gr. 1 than other group.

DISCUSSION

Increased broiler growth performance on increasing level of dietary SWP is supported by many previous finding (Chaudhary et al., 1998; Hossain et al., 1993; Borthakur and Sharma et al., 1998; Nandeesha et al.,1989;

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Jayaram and Shetty, 1980; Rahman, 1990; Shyma and Keshavanath, 1993; Begum, 1992; Rahman et al., 1996; Mahata et al., 1994). Improved feed conversion of broiler or diets with SWP in the current study coincides with finding of Venkatchalam et al. (1997) and Ling (1967).

In the present investigation, resultant data on important variables viz. live weight gain, feed consumption are presented in Table 2. Body weight gain at 8 week age of broiler chicks was found highest in the treatment Gr-3 as compared to other treated groups (50% FM + 50% SWPM). This supports the work of Das and Saikia (1972) and Horie and Watanabe (1980).

Table 3 - Mean body weight gain of broiler chicks in g/day

	Gr-1	Gr-2	Gr-3	Gr-4	Gr-5
Mean weight gain (g/day)	25.5	24.92*	26.73**	24.28***	23.58****
	25.9	24.99*	26.78**	24.34***	23.56****
	26.1	24.96*	26.77**	24.65***	23.59****
	25.3	24.98*	27.00**	25.02***	24.00****
	25.00	25.00*	26.00**	24.33***	23.22****
	25.56	24.97*	26.65**	24.52***	23.59****

*$F > F^{crit}$ as compared to Gr-1 (with values 8.746 and 5.318 respectively) ; **$F > F^{crit}$ as compared to Gr-1 (with values 17.478and 5.318 respectively); ***$F > F^{crit}$ as compared to Gr-1 (with values 18.116 and 5.318 respectively); ****$F > F^{crit}$ as compared to Gr-1 (with values 70.69and 5.318 respectively)

The efficiency of feed conversion was highest in Gr-3 dietary combination conforming the findings of Sengupta et al. (1995). No significant difference in survivability was found which also coincides with the findings of Das and Saikia (1972) and Sengupta et al. (1995). This is also supported by the fact that the Gross Growth Efficiency, Net Growth Efficiency and Assimilation efficiency was found to be quite satisfactory when chicks were fed with meal containing 50% SWP as evident from Table 4.

Table 4 - Energy Budget of broiler chick fed on formulated diet containing SWP & Commercial feeds

Feed group	Initial weight (g) W_1	Final weight (g) W_2	Production (g) $P = W_2 - W_1$	Total Food consumption (g) C	Average Feacal output (g) F	Assimilation $A = C - F$	Metabolism R $= P - A$	Assimilation efficiency A/C %	Growth ross efficiency $K_1 = P/C$ %	Net growth efficiency $K_2 = P/A$ %
Gr 1	45.16	1573.8	1528.64	1011.38	14.6	996.78	531.86	98.55	151.14	153.36
Gr 2	47.6	1543.52	1500.12	988.2	15.04	973.16	526.96	98.48	151.80	154.15
Gr 3	48.02	1576.4	1528.38	994.3	14.06	980.24	548.14	98.59	153.71	155.92
Gr 4	45.4	1532.22	1486.82	980.27	14.98	965.29	521.53	98.47	151.67	154.03
Gr 5	47.6	1462.02	1414.42	950.38	15.10	935.28	479.14	98.41	148.82	151.23

Table 5 - Comparative cost of feed formulation of different Experimental Group

Items	Rs/Kg	Gr 1		Gr 2		Gr 3		Gr 4		Gr 5	
		Kg	Cost	Kg	Cost	Kg	Cost	Kg	Cost	Kg	Cost
Maize	5.4	27.73	149.74	27.73	149.74	27.73	149.74	27.73	149.74	27.73	149.74
G.N.C.	12.5	10.66	133.25	10.66	133.25	10.66	133.25	10.66	133.25	10.66	133.25
Fish meal	58	3.88	225.04	2.91	168.78	1.94	112.52	0.97	56.26	0	–
SWP	Free	–	–	0.97	–	1.94	3.88	2.91	–	3.88	–
Rice polish	6	16.32	97.92	16.32	97.92	16.32	97.92	16.32	97.92	16.32	97.92
Wheat bran	7	1	7	1	7	1	1	1	7	1	7
Bone meal	40	0.5	20	0.5	20	0.5	20	0.5	20	0.5	20
Min. Mix.	76	0.5	38	0.5	38	0.5	38	0.5	38	0.5	38
Salt	9	0.25	2.25	0.25	2.25	0.25	2.25	0.25	2.25	0.25	2.25
Total feed cost			673.2		616.94		558.56		504.42		445.91

Thus, cheaper silkworm powder meal (SWPM) may be a supplement and has potential to replace the costly protein meal used in poultry industry. There was no mortality recorded in any group, which is also supported by Sengupta et al., (1995) and Das and Saikia (1972) who reported that mortality did not increased with SWP feeding. Early death of a few chicks was recorded in Gr-4 and Gr-5 due to cold winter weather of Ranchi. Post mortal investigation did not show any pathological symptoms. This indicated that SWP is not toxic to birds. This is also supported by the fact that there was no toxicological effect on broiler chicks and there may be some unidentified growth factors in SWP which have contributed to the better growth of broilers (Horie and Watanabe, 1980).

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The economics of the feed cost and broiler production is shown in Table 5. Profit was significantly higher as the level of dietary SWP was increased. These finding also coincide with the finding of Chaudhary et al. (1998), Rahman et al. (1996), Nandeeshi et al. (1989), and Habib and Hasan, (1995). They reported that SWP can be useful economical protein rich feed and can reduce production cost when F.M. is replaced by SWP

The feed efficiency was calculated by taking into account the total gain in live weight as well as total diet intake for the whole feeding trial period of 61 days. The cost of different diets was calculated on the basis of the prevailing market prices of feed ingredients and SWP which are free of cost. The total cost of feed and SWP in unit gain in live weight was calculated on the basis of quantity of feed consumed for one kg (Table 5). The cost benefit analysis was also calculated and it was found that the profit margin was highest for Gr.3.

CONCLUSION

The ingredients and dietary level of protein have received much attention of nutritionists because of the nutritional efficiency of protein. Feed cost per kg at 8 week of age gradually declined on increasing dietary level of SWP. Many authors concluded that the average weight gain is directly related to the level of protein in the diet. Hence this cheap waste product of Tasar silk industry can be effectively used as the replacement of costly, usually contaminated, fish meal as protein source in poultry feed.

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EFFECT OF TARTARIC ACID ADDITION ON RUMEN FERMENTATION, METHANE PRODUCTION AND DIGESTIBILITY IN DIFFERENT DIETS CONTAINING WHEAT STRAW *IN VITRO*

S.K. SIROHI*, P. PANDEY, N. GOEL, M. MOHINI, S.S. KUNDU

Dairy Cattle Nutrition division, National Dairy Research Institute, Karnal – 132001 Haryana, India

*E-mail: sirohisk@gmail.com

ABSTRACT: *The aim of the current study was to evaluate the effect of tartaric acid addition in diets on in vitro methanogenesis and rumen fermentation. Different levels of tartaric acid (5, 10, and 15 ppm) were tested for their effect on methanogenesis, rumen fermentation and digestibility in three wheat straw containing diets i.e. Low fiber diet (LFD, 40R:60C), medium fiber diet (MFD, 50R:50C) and high fiber diet (HFD, 60R:40C). Evaluation of tartaric acid was carried out using in vitro gas production technique. Methane production and individual fatty acids were estimated by Gas Chromatography. Results of different levels of tartaric acid on in vitro methanogenesis indicated that the maximum methane reduction (22.60% in term of mM/gDM) was observed in LFD at the supplementation dosage of 15 mM and a similar trend was seen, when methane was expressed in ml/gDM. Non-significant (P≤0.05) effect of tartaric acid addition on in vitro dry matter digestibility (IVDMD) was observed in almost cases. Protozoal population decreased with increasing concentration of tartaric acid and maximum reduction (54.64%) was in the MFD. Acetate to propionate ratio was decreased in tartaric acid supplemented diets which reflects increase in propionic acid production in comparison to control diet. Microbial biomass yield also increased due to the addition of tartaric acid in most of the diets.*

Key words: *Tartaric acid; Rumen fermentation; IVDMD, Microbial biomass; Methane production*

ORIGINAL ARTICLE

INTRODUCTION

Methane is one of the major end products of anaerobic fermentation of feeds in the rumen. Nutritionally, ruminal methanogenesis is a wasteful process which represents 2 to 12% gross energy loss from a mature animal (Moss, 1993). Methane production by animals, mainly from ruminants, is estimated to constitute 15 to 20% of the global production of methane (Crutzen et al. 1986). Its emissions to the atmosphere may result in a detrimental impact on the environment because of its greenhouse effect. Therefore, extensive research interests of animal nutritionists and ruminant microbiologists have been focused on developing methods of reducing methane production and manipulate the ruminal microbial ecosystem to improve the feed conversion efficiency. Many strategies such as processing of forages (Takahashi, 2001; Santoso et al., 2003), increasing the proportion of concentrates in the diet (Lee et al. 2003), and supplementation of some methane inhibitors such as halogenated compounds (Martin and Macy, 1985), ionophores (Van Nevel and Demeyer, 1988), organic acids (Martin, 1998), sarsaponin (Lila et al. 2003), and unsaturated fatty acids (Czerkawski et al., 1966) have been proposed as a means of reducing methane production in the rumen. Another method to reduce the methane formation in the rumen is diverting H_2 from CH_4 production to increase alternative electron sink metabolic pathways to dispose of the reducing power (Lopez et al. 1999; Ungerfeld et al. 2003 and Newbold et al. 2005). Dicarboxylic acid can act as alternative hydrogen sinks in the formation of methane in enteric fermentation; consequently, they are precursors to propionate production in the rumen. Fumarate and malate are the key propionate precursors in the dicarboxylic acid pathway (Castillo et al., 2004) and may act as a hydrogen acceptor (Martin and Park, 1996) hence both malate and fumarate have been increased pH, total volatile

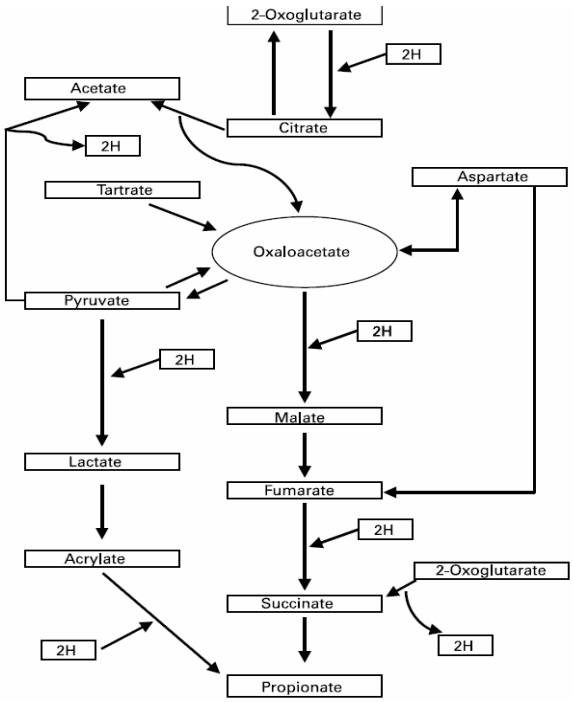

Fig 1- Possible fermentation pathway for tartaric acid (Newbold et al. 2005)

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fatty acid production and concentration of propionate in the rumen (Martin and Streeter, 1995; Carro and Ranilla, 2003). Tartaric acid is also converted into oxaloacetate and enters into TCA cycle and converted to propionic acid (Figure 1).The objective of the present study was to examine the effect of the addition level of tartaric acid in rumen fermentation characteristics and methane production by rumen microbes *in vitro*.

MATERIALS AND METHODS

Feeds and experimental design

To evaluate the response of Tartaric acid three diets were prepared by taking different roughage and concentrate ratio i.e. High fiber diet (HFD, 60R:40C), medium fiber diet (MFD, 50R:50C) and low fiber diet (LFD, 40R:60C) and milled to pass through a 1 mm sieve and used as substrate. The roughage part composed of wheat straw and the concentrate part composed of maize (33%), GNC (21%), mustard cake (12%), wheat bran (20%), de-oiled rice bran (11%), mineral mixture (2%) and salt (1%) respectively. Tartaric acid (Sigma-Aldrich, EC203-743-0) was added in incubation medium to achieve a final concentration of 0, 5, 10 and 15 mm. All the treatment combinations were arranged in 4 x 3 factorial design with three replicates. A set was incubated devoid of substrate with and with out tartaric acid which served as blanks for a particular treatment and values were corrected for different parameters with these blanks.

Preparation of inoculums and *in vitro* gas production

Rumen liquor was collected after manual mixing of rumen contents from a fistulated mature male buffalo (*Bubalus bubalis*) maintained on a standard diet (60 parts roughage: 40 parts concentrate) before the morning feeding into a pre-warmed insulated flask and brought into the laboratory. Permission has been taken from Animal ethics committee of institute for taking rumen liquor from male fistulated buffalo. The rumen liquor filtered through four layers of muslin cloth and then the required amount of filtered rumen liquor used as a source of inoculum. The incubation medium was prepared as per previously described method (Menke and Steingass, 1988).

The tartaric acid solution was injected as per the dose by small syringe into 100 ml glass syringe containing 200±10 mg of milled (1mm) three type wheat straw based diets. The 30 ml incubation medium was dispensed anaerobically in each syringe. The plungers of syringes applied with petroleum jelly for smooth movement and stop any leakage. Syringes were closed using clamps and were incubated at 39±0.5^{0}C for 24 h.

Estimation of methane production by Gas Chromatography

Methane content in fermentation gas was determined by gas chromatography (GC) using Nucon-5765 gas chromatograph described by (Sirohi et al., 2012). For methane estimation, each gas sample (250 µl) was manually injected using Hamilton airtight syringe. Methane content in the sample was calculated by external calibration, using a certified gas mixture with 50% CH_4 and 50% CO_2 (Spantech calibration gas, Surrey, England). The peak of methane gas was identified on the basis of retention time of standard methane gas and the response factor obtained was used to calculate methane percentage in the gas sample. The methane produced from substrate during 24 h incubation was compared to the blank values. The volume of methane produced was calculated as follows: Methane production (ml) = Total gas produced (ml) × % methane in the sample.

Rumen fermentation parameters

TVFA concentration (mM/100 ml) in the supernatant was estimated according to prescribed method (Barnet and Reid, 1957). For the estimation of IVFA, 1 ml of the supernatant was treated with 25% meta-phosphoric (4 ml) and kept for 3-4 h at ambient temperature (Erwin et al. 1961). Thereafter, IVFA was estimated using gas chromatograph according to the prescribed method (Sirohi et al. 2012). Sample (2 µL) was injected through the injection port using Hamilton syringe (10 µL). Individual VFAs of the samples were identified on the basis of their retention time and their concentration (mmol) and calculated by comparing the retention time as well as the peak area of standards after deducting the corresponding blank values. For the estimation of ammonia nitrogen, the supernatant of each syringe including that of blank was used for NH_3-N estimation. Supernatant (5 ml) was mixed with 1 N NaOH (12 ml) and steam passed on this using KEL PLUS-N analyzer (Pelican, India) and the NH_3 evolved was collected in a boric acid solution having a mixed indicator and titrated against N/100 H_2SO_4.

Partitioning factor (PF) and microbial biomass yield (MBM)

The PF is calculated as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it. Substrate provides important information about partitioning of fermentation products. The MBM yield was calculated by using the degradability of substrate and gas volume and stoichiometrical factor (Blummel et al., 1997).

Microbial biomass (mg) = Substrate truly degraded - (gas volume × stoichiometrical factor) Where the stoichiometrical factor used was 2.25.

Protozoa counting

For protozoa count one milliliter of the fermentation fluid was diluted with 1 ml of formalin (18.5% formaldehyde) and 3-4 drops of brilliant green and then incubated for 24 h at room temperature. The stained

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protozoa were diluted (if needed) and counted by haemocytometer as per the method described by Dehority (1984).

In vitro true DM degradability

To estimate true DM degradability of feed sample of each syringe containing residues after incubation was estimated as per the prescribed method (Van Soest et al., 1991).

Proximate analyses and Cell wall constituents

The proximate analysis of substrate was carried out as per the methods (AOAC, 1995). The cell wall constituents of substrates were determined according to the described method (Van Soest et al., 1991).

Statistical analysis

Experimental data was fitted into 3x4 factorial arrangement for different parameters and analyzed in complete randomized block design with three replicates for analysis of variance (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

The physical and chemical composition of all the three wheat straw based diet was shown in Table 1. The effects of tartaric acid addition on *in vitro* rumen fermentation pattern and methane production of different diets were shown in table 2 and 3, respectively.

Table 1- Physical and chemical composition diets used as substrates in *in vitro* incubations

Ingredient of diets		
Diets	G/kg on DM basis	
	Wheat straw	Concentrate
HFD	600	400
MFD	500	500
LFD	400	600

Ingredient of concentrate	
Particulars	G/kg on DM basis
Maize	330
Ground nut cake	210
Mustard cake	120
Wheat bran	200
Deoiled rice bran	110
Mineral mixture	20
Salt	10

Chemical constituents of diets (g/kg on DM basis)							
Diets	OM	CP	EE	NDF	ADF	HC	TA
HFD (60R:40C)	867.6	108.6	23.4	623.1	372.0	251.1	132.4
MFD (50R:50C)	878.4	125.3	30.4	604.5	329.5	275.0	121.6
LFD (40R:60C)	875.6	142.7	34.8	538.7	298.7	240.0	124.4

HFD: High fiber diet, MFD: Medium fiber diet, LFD: Low fiber diet, OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, HC: Hemicelluloses, TA: Total Ash

Table 2 - Effect of tartaric acid on rumen fermentation pattern in different diets *in vitro*

Diets	Dose (mM)	IVDMD (%)	PF	MBM (mg)	CH_4 (ml/gDM)	CH_4 (mM/gDM)	Protozoa (x10^4/ml)
HFD	0	55.67	2.24	63.92	38.53	3.83	1.67
	5	57.83	2.71	75.08	32.83	3.26	1.00
	10	51.83	2.74	68.08	41.41	4.12	1.00
	15	53.67	3.77	80.92	32.06	3.19	0.83
MFD	0	57.83	2.23	66.08	42.70	4.25	1.83
	5	58.67	2.69	75.92	38.08	3.79	1.17
	10	56.00	2.67	72.25	37.06	3.69	1.17
	15	55.67	3.63	82.92	33.40	3.32	0.83
LFD	0	64.67	2.21	72.92	40.94	4.07	1.50
	5	63.00	2.63	80.25	37.69	3.75	1.50
	10	66.33	2.54	82.58	40.65	4.04	1.00
	15	65.83	3.25	93.08	31.67	3.15	1.00
SEM	D	1.04	0.03	1.04	NS	NS	NS
	T	NS	0.03	1.20	0.91	0.09	NS
	D*T	NS	0.06	NS	NS	NS	NS

HFD: High fiber diet, MFD: Medium fiber diet, LFD: Low fiber diet, PF: Partition factor, MBM: Microbial biomass yield, CH_4: Methane, SEM: Standard of Means, D: Diets, T: Dose

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Table 3 - Effect of tartaric acid on rumen fermentation pattern in different diets *in vitro*

Diets	Dose (mM)	TVFA (mM/100ml)	Acetate (mM/100ml)	Propionate (mM/100ml)	Butyrate (mM/100ml)	A/P ratio	NH_3-N (mg/100ml)
HFD	0	8.23	5.31	2.70	0.22	1.97	14.75
	5	7.10	3.62	3.17	0.31	1.14	14.56
	10	4.68	2.31	2.18	0.19	1.05	15.40
	15	5.83	2.52	3.04	0.27	0.83	15.59
MFD	0	5.40	1.47	3.67	0.26	0.40	15.77
	5	5.58	2.67	2.65	0.26	1.01	15.87
	10	5.28	2.40	4.26	0.40	0.56	15.96
	15	7.03	2.80	3.78	0.44	0.74	13.53
LFD	0	9.05	4.97	3.99	0.19	1.25	19.23
	5	9.15	5.09	3.97	0.19	1.28	21.65
	10	6.38	4.42	4.67	0.32	0.95	21.93
	15	5.83	2.52	3.65	0.21	0.69	16.05
SEM	D	NS	0.26	NS	NS	0.22	0.34
	T	NS	0.31	0.27	0.03	0.25	0.40
	D*T	0.50	0.53	0.46	0.04	0.43	0.69

HFD: High fiber diet, MFD: Medium fiber diet, LFD: Low fiber diet, TVFA: Total Volatile Fatty Acid, A/P: Acetate to propionate Ratio, NH_3-N: Ammonia nitrogen, SEM: Standard of Means, D: Diets, T: Dose

In the current experiment, results of IVDMD was non-significantly ($P \leq 0.05$) affected due to the addition of tartaric acid. IVDMD values almost remained similar as control at all levels of tartaric acid supplementation and different types of diets. These results were more or less in accordance with previous studies. Increases in DM degradation were not observed when free acids were used (Newbold et al., 2005), while, small increase, approximately 4% in the apparent *in vitro* digestibility of maize supplemented with fumarate (Carro and Ranilla, 2003). The partition factor (PF) and microbial biomass production (MBM in mg) values were increased ($P<0.05$) with supplementation of tartaric acid at different concentration in all types wheat straw based diets. The highest increase 68.30, 62.78 and 47.06% in PF and the highest increase in MBM (mg) was 26.68, 25.48 and 27.65% found at 15 mM concentration, as compared to control in HFD, MFD and LFD, respectively. A reduction in methane production (ml/gDM, mM/gDM) was seen except 10 mM level in all cases, which however does not, reduced the methane production significantly. The maximum methane reduction was observed at highest level i.e. 15 mM in all diets. Results indicate that the highest methane reduction (16.71, 21.88 and 22.60%) was noticed at 15 mm level in HFD, MFD and LFD, when expressed in mM/gDM respectively. The similar trend was noticed when methane reduction was expressed in ml/gDM (table -2). Previous studies indicate that methane production was decreased with increase the concentration of free acid or propionate precursor (Asanuma et al., 1999; Lopez et al., 1999a; Iwamoto et al., 1999). In the present experiment, a reduction in protozoa number was also observed with the increasing concentration of tartaric acid. At the 15 mM dosage/concentration of tartaric acid, the maximum reduction in protozoa number was found i.e. 50.30, 54.64 and 33.33 percent in HFD, MFD and LFD, respectively. Tartaric acid supplementation showed non-significant ($P<0.05$) effect on TVFA concentration in comparison to control. Apparently slight changes in TVFA concentration were observed in all cases except in HFD at 10 mM level, whereas, maximum 43.13% reduction was observed, while, in case of MFD, the maximum increase (30.19%) was noticed at 15 mM level. Acetate concentration decreased in almost all cases except MFD, although the decrease was non-significant and maximum reduction (52.54%) was observed in HFD at 10 mM level. In case of MFD, it increased and maximum increased (47.50%) was noticed at 15 mM level. A slight effect of tartaric acid inclusion on propionic acid concentration (mM/100ml) was seen in all the three types of diets. Results indicated that the maximum increase (17.41%) was noticed in HFD at 5 mM level, and at the same level in MFD show the maximum reduction (27.79%) in propionic acid concentration. Slight change in butyrate concentration was also observed in the present study (table -3). In the present study, reduction in A/P ratio was observed in HFD and LFD in all concentration of tartaric acid, while in case of MFD, it was increased and highest increase (60.60%) was seen at 5 mM concentration. In the present experiment, slight change in NH_3-N concentration was observed due to tartaric acid supplementation. The concentration of ammonia nitrogen decreased with high level i.e. 15 mM in MFD and LFD, while increasing in HFD at the same level. The maximum reduction was (16.54%) found in LFD at 15 mM level. The results of the study was in accordance of results of Newbold et al. (2005) as they reported that with addition of organic acid in general able to decrease methane production due rechanneling the available hydrogen towards propionic acid production without affecting dry matter digestibility even with fumaric acid and malic acid supplementation digestibility was rather increased in some cases (Carro and Ranilla, 2003; Sirohi et al., 2012)

CONCLUSIONS

In the present study it was concluded that tartaric acid addition in wheat straw containing diets is able to significantly decrease the methane production by diverting the available hydrogen towards propionate production without affecting the digestibility, but more studies are required to validate the results under *in vivo* conditions.

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BIOMETRY AND TESTICULAR GROWTH INFLUENCED BY NUTRITION ON PREPUBERTAL PELIBUEY LAMBS

J.M. MARTINEZ[1,2], B. DOMINGUEZ[2]*, M. BARRIENTOS[2], R. CANSECO[2], E. ORTEGA[3], C. LAMOTHE[2]

[1]*Módulo de ovinos y caprinos, Rancho Torreón del Molino, km. 14.5 Carretera Federal a Xalapa, Veracruz. México. Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana. C.P. 91710*
[2]*Laboratorio de Radioinmunoensayo, Rancho Torreón del Molino, km. 14.5 Carretera Federal a Xalapa, Veracruz. México. Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana. C.P. 91710*
[3]*Colegio de Postgraduados-Campus Veracruz. km. 26.5 Carretera Federal a Xalapa, Veracruz. México*

*E-mail: beldominguez@uv.mx

ABSTRACT: *The growth and testicular development was studied in 48 Pelibuey male lambs 76.6±3.0 days of age and 12.7±1.9 kg body weight (BW), two groups were designed (n=24). 1: Intensive rotational grassing (IRG), 2: Intensive rotational grassing plus nutritional supplement (IRGS). BW was recorded every 15 days from 75 days of age to the onset of puberty. The animals grazed on Panicum maximum. IRGS received a concentrate with 15% of protein. The testicular biometry included scrotal circumference (SC) and testicular volume (TV). Blood samples were collected each 15 days from 90 to 190 days of age for evaluate the testosterone concentrations. BW, SC and TV at histological puberty was higher in IRGS than IRG; 22.5±1.5 vs. 16.06±1.5 kg, 22.0±1.0 vs. 12.2±1.5 cm, 60.5±1.7 vs. 12±3.5 cm^3 respectively (P<0.05) with an average age for the two groups of 162±7.0 days. The correlation coefficient (R) was higher (P<0.05) for SC vs BW than age vs BW (0.884 vs 0.816) and the TV vs. BW than TV vs. age (0.849 vs. 0.777) in the IRGS; the IRG showed lower R for the same comparisons (P<0.05). Seminiferous tubules showed lumen by day 142, spermatids and spermatozoids by day 171 for IRGS, meanwhile in the IRG only showed gonocytes and Sertoli cells. Testosterone concentrations reached a peak (2.5 ng/ml) at 168 days of age for the IRGS meanwhile the IRG showed lower levels than 0.05 ng/ml. Testicular development and testosterone concentrations depends more on BW than age; and they are modified by the nutritional management in prepuberal male lambs.*

Key words: *Testis Development, Puberty, Nutrition, Lambs*

ORIGINAL ARTICLE

INTRODUCTION

In sheep, as in any other domestic species, the reproductive performance is considered as the most important in terms of economic value (Bilgin et al., 2004). There are four factors that can determine performance: (1) genetic merit, (2) physical environment, (3) nutrition and (4) management; it has been suggested that the nutritional factors are the most important in terms of their direct effects on reproduction, while the other factors are considered as having only irregular influence (heat stress, pre weaning management, for example). Adequate nutrition can stimulate biologically mediocre individuals to attain their genetic potential, diminish the negative effect of a physically hostile environment and minimize the effects of deficient management techniques (Fourie et al., 2004). Therefore, appropriate nutritional management is a decisive factor for the successful reproduction of a flock (Fernández et al., 2004); indeed, the energy deficiency caused by a low level of ingestion or by excessive utilization diminishes the secretion of gonadotrophines in both sexes of many species, humans included, but reestablishing normal feeding patterns generally reverses any deficit of hormones (Brown, 1994; Blache et al., 2000; Bielli et al., 2002). Testicular size and sperm production may be affected by changes in protein ingestion, even when such changes exceed the maintenance requirements (Fernández et al., 2004). There appears to be no reciprocal effect from changes in the secretion of testosterone, and this tends to strengthen the hypothesis that the connection between protein ingestion and reproduction is based on an effect not dependent on GnRH (Hötzel et al., 1998; Fernández et al., 2004). However, it has been established that the regulation of testicular growth through nutrition also includes a route that is dependent on GnRH (Blache et al., 2000). Numerous studies (Blache et al., 2000; Fourie et al., 2004) have shown that spermatogenesis in rams is sensitive to increments in protein ingestion. This effect has been associated with an increase in testicular size as reflecting an increase in the volume of the

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seminiferous epithelium and in the diameter of the seminiferous tubuli (Saab et al., 1997; Hötzel et al., 1998); thus, the size of the testis is directly related to the potential of sperm production.

In the case of rams, changes in body weight (BW) are directly correlated with testicular growth and regression (Murray et al., 1990). The size of the testis is considered as the most adequate criterion, from the physiological, genetic and practical perspective, for improving the reproductive performance of female descendents; this indirect criterion of selection is dependent on the heritability and the genetic correlation between testicular size and female reproductive traits (Matos et al., 1992). It has been observed that males with bigger testis tend to produce daughters that reach puberty at an earlier age and liberate more ovules during each estrous period (Söderquist and Hultén, 2006).

Concerning the nutrition of growing rams, it has been reported that the reproductive functions in young animals seems to be more susceptible to restrictions in energy and protein than those in adults; furthermore, severe nutritional restrictions can result in permanent damage to gonad and neural tissues (Brown, 1994). Recently, Bielli et al. (2002) reported that, starting in the uterine stage, deficient nutrition during pregnancy in the ewe may reduce testicular development in the newborn lamb, although poor nutrition and the ingestion of toxic substances can have a greater effect on testicular development and spermatogenesis. The reproductive system possesses considerable regenerative capacity, unless there have been severe and prolonged dietary deficiencies (Brown, 1994). Post-weaning nutritional management strongly influences weight increase in rams, which has been found to be associated with testicular growth and the onset of puberty in rams of the Menz breed (Mukasa-Mugerwa and Ezaz, 1992); besides, measuring the scrotal circumference (SC) is an essential characteristic of andrological evaluation if we take into account that testis size varies according to the breed, the age and the season of the year (Söderquist and Hultén, 2006). Therefore, measuring the size of the scrotum as a criterion for early selection in small ruminants (Mukasa-Mugerwa and Ezaz, 1992) makes it possible to measure the performance of these rams bred under different nutritional strategies and, consequently, to evaluate their diets as inductors of precocity. The object of this study was to determine the correlations between age and BW with testicular biometry, in addition to measure the testosterone concentrations and histologically determine the presence of spermatozoids in prepubertal Pelibuey rams under two nutritional regimes: intensive rotational grazing and intensive rotational grazing plus nutritional supplement.

MATERIALS AND METHODS

Location

This research project was carried on for one year at the Postgraduate College – Veracruz Campus, situated at 19°11'45" N and 96°19'03" W (GPS 12, Garmin International Inc.), in a warm climate with rains in summer.

Experimental animals

Forty-eight Pelibuey male lambs aged 76.6±3.0 days with BW 12.7±1.9 kg, born from single or double parturition with twins of either sex, were randomly assigned to either of two experimental groups (n=24). Group 1: intensive rotational grazing (IRG), Group 2: intensive rotational grazing plus nutritional supplement (IRGS); each group consisted of 8 weaned lambs during each of the following three climatological seasons of the year: rainy: August to November; windy: December to March; dry: April to July. The BW was recorded every 15 days from 75 days of age to the onset of puberty in any lamb identified with the aid of histological techniques.

Feeding

The animals grazed in established meadows of Tanzania grass *(Panicum maximum)* for 7 days, followed by 21 days of rest for each meadow. The IRGS group received a commercial concentrate for lambs (Campi corderos®, Veracruz, Mexico) with 15% of crude protein, the supply of which was adjusted to be equivalent to 1.5% of the BW recorded on the scales every 15 days during the 3 seasons of the year.

Morphological evaluations

A biometry was performed in the testicular region as follows: (1) The scrotal circumference (SC) was obtained by forcing both testicles to descend completely into the scrotum (Matos et al., 1992), with the aid of a flexible measuring tape placed at the maximum transverse diameter encountered in the scrotal sac (Bielli et al., 2000). The testicular volume (TV) was calculated from the biometry performed at the greater and lesser axes of each testis with the aid of a vernier graduated in millimeters; for this calculation, the following equation proposed by Steger and Wrobel (1994) was applied:

$$TV = 1/6 * (\pi) * a * b * 0.945 \quad \text{(Equation 1)}$$

In which:
TV=testicular volume
π=3.1416
a=testicular width
b=testicular length

The testicular biometry was performed at intervals of 15 days, starting from the 75th day of age in all the animals of both feeding groups and in all seasons of the year.

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Histological evaluations

In order to obtain testicular samples, a hemicastration by means of lateral approximation was performed at intervals of 15 days starting on day 90 of age. For these evaluations only one animal from each feeding system and season of the year was employed. The animals were tranquilized with a sedative consisting of xylazine (Rompun® Bayer) and ketamine (Ketamina® Cheminova). After castration, a sample of tissue in the form of a cube with a volume of 8-10 mm^3 was taken from each of the three transverse sectors at the greater testicular axis, and then the samples were fixed in a modified Davidson solution (Latendresse et al., 2002) during 48 hours. Thereafter, they were washed in ethyl alcohol at 70% for two hours on two occasions; in this last solution they were processed to obtain histological sections of 5 µm in thickness for their subsequent staining with hematoxylin and eosin. Once the stained laminas had been obtained, they were examined under a microscope (Leica microscope 40X) for the cellular development and structures, such as: (1) the lumen, (2) spermatocytes, (3) spermatids or spermatozoids, so as to have a histological basis for determining the onset of puberty, such as the initial liberation of spermatozoids from the seminiferous epithelium (Herrera-Alarcón et al., 2007). The images were taken with the help of a Motic 1.3 Mpxel digital camera.

Endocrinological evaluations

Blood samples from the jugular vein were collected in tubes having an anticoagulant (EDTA) at 15-day intervals between 90 and 210 days of age during the rainy season. The samples were centrifuged at 2000 g for 10 minutes, and the supernatant plasma was recovered and frozen at -20 °C for its quantification. The testosterone concentration was determined by solid phase radioimmunoassay with a commercial antibody kit marked with I^{125}, and the reading was taken with an automatic gamma counter (2470 WIZARD2, Perkin Elmer) (Ungerfeld and Silva, 2004).

Statistical analysis

The data were recorded on an electronic calculation sheet. The age (in days) and the body weight were used as independent variables, the dependent variables were: scrotal circumference (cm) and testicular volume (cm^3). To measure the degree of association, a simple exponential equation was employed:

$$f = a * e\wedge(b * x)$$ (Equation 2)

In which:

f = dependent variable (SC cm or TV cm^3)

a = value of the body weight to the maturity (estimated)

e = 2.7182

b = value of curve integral

x = age (days) or weight (kg)

To describe the growth curve of the lambs, a Gompertz mathematical model was used:

$$BW = a * e\wedge(-e(-(x - x_0)/b$$ (Equation 3)

In which:

BW = body weight (kg)

a = value of the body weight to the maturity (estimated)

b = inflection point in days (age when maximum growth is observed)

x = age (days)

x_0 = age at initial curve inflection

To measure the relationship between scrotal circumference (cm) and testicular volume (cm^3), the following equation was used:

$$SC = y_0 + a * \ln(x)$$ (Equation 4)

In which:

SC = scrotal circumference (cm)

y_0 = value of scrotal circumference (cm) when testicular volume (cm^3) is zero

a = integral of equation

x = testicular volume (cm^3)

RESULTS AND DISCUSSION

The results show that the quality of the diet is a determining factor in body development (Fig. 1e) and hence in testicular growth (Figure 2); this is similar to observations Fourie et al. (2004), reported for young Dorper rams, in which a better diet with greater energy and protein complementation was able to improve reproductive performance. In previous work with adult rams of the Assaf breed (Fernández et al., 2004), statistical differences in testicular size and sperm production were found upon comparing diets with different protein contributions: the values recorded for SC and TV were lower in sheep that consumed barley chaff and a nutritional supplement with 13.6% of crude protein (CP) than in those that received supplements containing 16.4% and 20.5% of CP concentrate (Fernández et al., 2004). Bielli et al. (1999), failed to find any significant effects on testicular dimensions upon improving the forage or increasing the protein in the diet for rams of the Corriedale breed. In

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Merino rams, however, it was found that testicular dimensions responded better to the ingestion of digestible energy than to the availability of CP in the diet, which produced only a marginal effect (Murray et al., 1990). In the present study, the experimental animals that consumed a better diet (IRGS group) showed more gonadal growth (Table 1); this made it possible for them to reach histological puberty at ages oscillating between 156 and 177 days during all the climatic seasons studied. The BWs recorded were between 22 and 23 kg, which are similar to those reported by Herrera-Alarcón et al. (2007) for rams of the Blackbelly breed.

Table 1. Biometric descriptors of Pelibuey rams kept in conditions of intensive rotational grazing (IRG), or rotational grazing plus nutritional supplement (IRGS) at the moment of histological puberty were determined in the IRGS in different seasons of the year

Biometric Descriptor	Intensive Rotational Grazing Plus Supplement (IRGS)			Intensive Rotational Grazing (IRG)		
	Rainy	Windy	Dry	Rainy	Windy	Dry
Body weight (kg)	22.5	23.0	22.0	15.5±1.05	16.3±0.98	16.4±1.0
Scrotal circumference (cm)	23.0	22.0	21.0	9.4±0.4	13.1±0.3	14.2±0.4
Testicular volume (cm^3)	62.0	61.1	58.4	1.9±0.2	12.2±1.95	22.6±2.35
Age of puberty (days)	177	156	166	167±6.0	145±7.0	164±9.0

Note: Values for the IRGS were obtained from the first animal to attain histological puberty; the number of animals in the IRG was 4.

It has been reported that, in rams of the Chios, Serres and Karaguniki breeds born in the month of October, the first spermatozoids appear in the ejaculate around 142 days of age, when their weight averages 35 kg (Alexopoulos et al., 1991). Puberty in rams is considered to begin at the first mounting with ejaculation and appears to be associated more with BW than with chronological age (Belibasaki and Kouimtzis, 2000). Fernández et al. (2005) reported that the SC was smaller in rams fed with a nutritional supplement having low protein content than in those receiving a supplement high in protein, which improves the performance in the animals during the mating period and accelerates testicular growth. In the IRG group, differences occurred in the testicular biometry during the various seasons of the year (Table 1); these may be attributable to fluctuations in the quality of forage during the experimental phases in the field.

Mukasa-Mugerwa and Ezaz (1992) found significative reproductive variations due to effects arising from the season of birth, nutrition level and weight at weaning. In the present study, the SC at the beginning of puberty showed an average of 22±1 cm for the IRGS group in all seasons. In a study carried out by Avellaneda et al. (2006), the SC at the onset of puberty was 23.8, 22.9, 20.8 and 23 cm for the Romney Marsh, Mora Colombian, Creole and Hampshire breed, respectively. In the present study, TV was 60.5±2 cm^3, at an age of 166±11 days and with a BW of 22.5±1 kg (Table 1). Similar values were founded when evaluating the testicular characteristics of Ile de France x Akkaraman rams, with average SC measurements of 23.8±0.55 cm and TV of 51.7±2.76 and 57.8±3.76 cm^3 for the left and right gonads, respectively (Mert et al., 2009). Nevertheless, these values are lower than those reported by Herrera-Alarcón et al. (2007) from their work with Blackbelly rams aged 172 days; they recorded a value of 33.5 cm for the SC in rams of mature age. According to Söderquist and Hultén (2006), the SC in rams of the Gotlandic breed measured 28.9±1.9 cm at an age of 170±9 days, while the BW was found to be 53.5±7.0 kg for Merino rams at 73 and 143 days, Steger and Wrobel (1994) reported TV of 7.18 and 149.13 cm^3, respectively. In the present work, a marked difference was found to exist upon analyzing the TV of both gonads and comparing ages; a correlation coefficient of R=0.777 was obtained for the IRGS group, and R=0.092 for the IRG group (Table 2, Figure 1c).

Table 2 - Adjustments in the testicular biometry performed on Pelibuey rams kept in conditions of rotational grazing (IRG), or rotational grazing plus nutritional supplement (IRGS)

	Intensive Rotational Grazing plus Supplement (IRGS)				Intensive Rotational Grazing (IRG)			
1) Body weight (kg)	a	b	R	R^2	a	b	R	R^2
Scrotal circumference (cm)	4.08±0.220	0.070±0.003	0.884	0.780	4.27±0.332	0.06±0.005	0.711	0.503
Volumen testicular (cm^3)	0.44±0.126	0.023±0.013	0.849	0.720	0.30±0.129	0.24±0.021	0.653	0.423
2) Age								
Scrotal circumference (cm)	5.81±0.30	0.006±0.004	0.816	0.664	9.44±0.73	0.001±0.0006	0.189	0.029
Testicular volumen (cm^3)	1.91±0.41	0.020±0.001	0.777	0.602	7.00±3.35	0.003±0.003	0.092	0.002
Body Weight (kg)	31.82±8.28	127.37±53.0	0.770	0.593	15.27±0.47	23.36±11.92	0.340	0.115
3) Testicular Volume (cm^3)	a	y_o	R	R^2	a	y_o	R	R^2
Circunferencia escrotal	3.35±0.07	5.23±0.211	0.962	0.926	2.75±0.09	6.41±0.188	0.921	0.848

a, b = adjustment parameters for the mathematical model; y_o= value of intercept, R= correlation coefficient, R^2= determination coefficient.

In Menz lambs, the age and the BW at the start of puberty were 288±6 days and 19.3±0.4 kg, respectively, the SC being 21.5±0.3 cm (Mukasa-Mugerwa and Ezaz, 1992). Regarding other breeds, the BW at puberty was 31.2, 29.0, 26.9 and 29 kg, at the ages of 235, 214, 231 and 196 days for the Romney Marsh, Mora Colombian, Creole and Hampshire breed, respectively (Avellaneda et al., 2006). In a study carried out under semi-cold and semi-humid climatological conditions in Mexico, Pelibuey lambs reached puberty at 144.07±8.43 days with a BW of 32.6±3.94 kg and an SC of 25.86±2.24 cm, on a diet that contained 2.85 Mcal of metabolized energy/kg

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(Valencia et al., 2005). By utilizing the point of inflection on the growth curve for the SC, ages of 140 and 152 days at puberty were determined for the Redkaraman and Awassi breeds, respectively (Emsen, 2005); whereas for lambs of the Friesland, Karagoniki, Chios and Serres milch sheep breeds, puberty was determined as starting at 170,187, 189 and 209 days, respectively (Belibasaki and Kouimtzis, 2000).

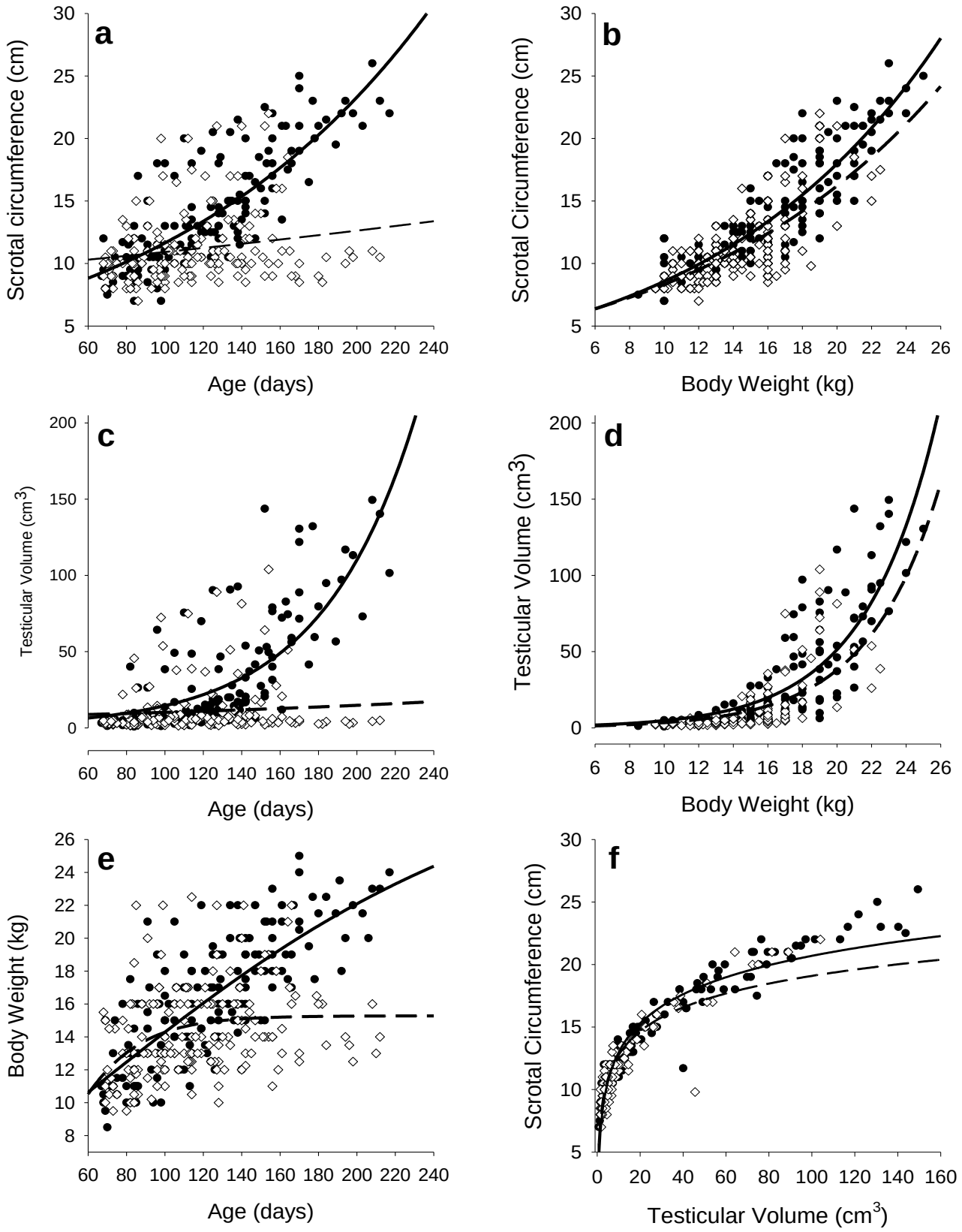

Figure 1 - Adjusted curves of the testicular biometry in regard to age (a,c) and body weight (b,d). Solid line: Intensive Rotational Grazing Plus Supplement (IRGS); Broken line: Intensive Rotational Grazing (IRG). ●IRGS, ◇IRG. **e**: age *vs*. bodyweight relationship; **f**: testicular volume *vs*. scrotal circumference relationship

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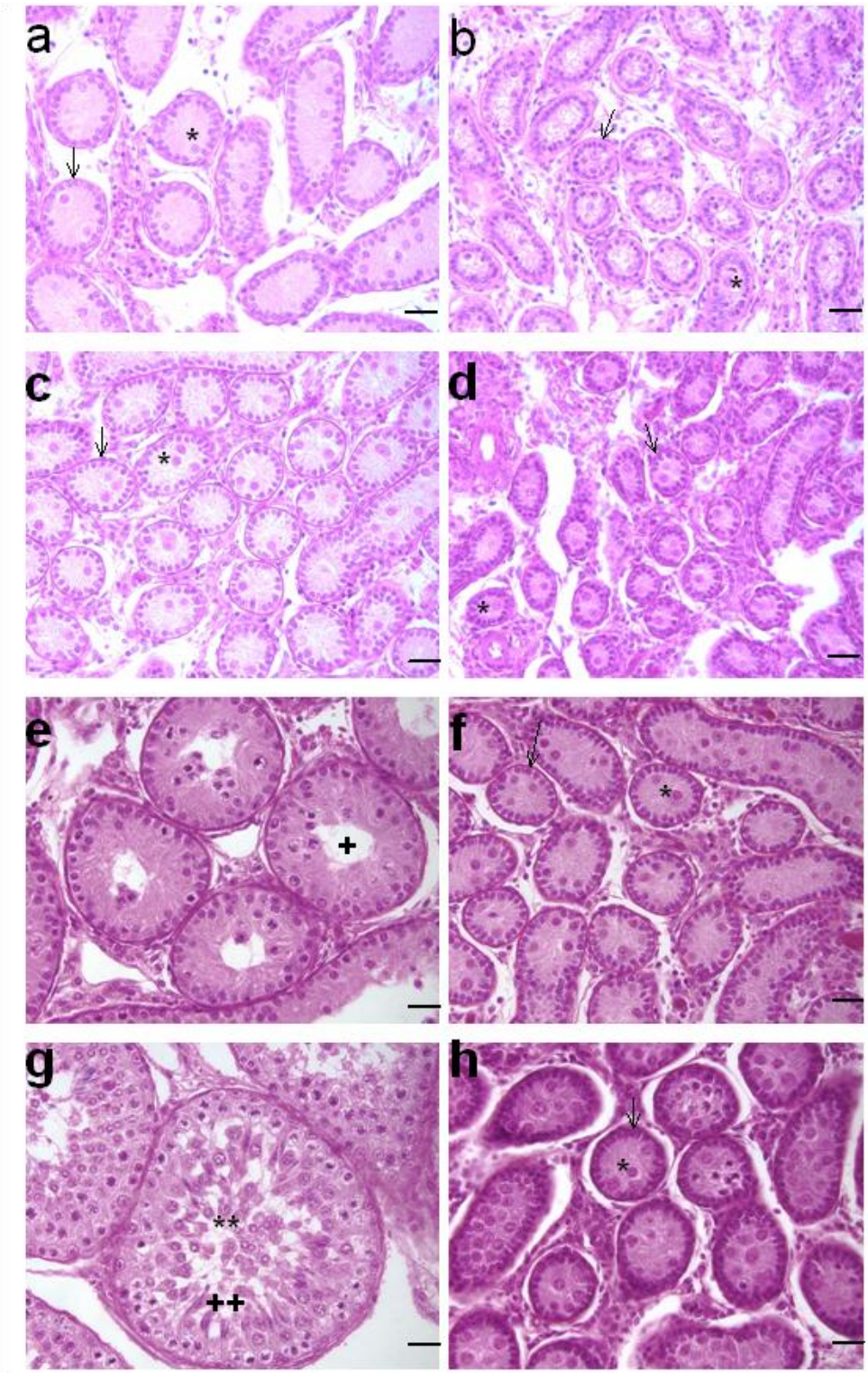

Figure 2 - Findings in the germinal testicular epithelium (40x). **a,c,e,g:** Intensive Rotational Grazing plus Supplement (IRGS); **b,d,f,h:** Intensive Rotational Grazing (IRG). **a,b:** age 83±2 days; **c,d:** age 113±1 days; **e,f:** age 142±1 days; **g,h:** age 171±5 days; mean ± standard deviation. *Prespermatogonium (Gonocyte); →Sertoli cells; +Lumen of seminiferous tubule; **Spermatides; ++Spermatozoids. The bar in the lower right corner of all pictures measures 25 µm

In this study, the correlation coefficient between BW and SC was R = 0.884 for the IRGS (Table 2); this coincides with the correlation value (R=0.86) reported for the testicular growth and changes in the BW of Merino lambs (Murray et al., 1990) and that of R=0.85 for Menz lambs (Mukasa-Mugerwa and Ezaz, 1992). Considering the high correlation between the BW and the SC, it is necessary to evaluate puberty on the basis of body and gonadal development rather than age (R=0.816), since the SC is a direct indicator of sperm quality (Avellaneda et al., 2006). The correlation between BW and SC for the IRG was R=0.711 in the present work (Table 2).

Values of R^2=0.780 and R^2=0.503 were recorded as determination coefficients for the IRGS and the IRG, respectively (Table 2, Figure 1b), and these reinforce the finding that testicular measurements increase progressively and are better correlated with the BW than with the age (Salhab et al., 2001).

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The correlation value for SC *vs* TV was R=0.921 for the IRG and R=0.962 for the IRGS. In lambs from the Ile de France x Akkaraman breeding, the correlation between the left SC and TV was R=0.84, and R=0.90 for the right (Mert et al., 2009). For these reproductive variables, determination coefficients of R^2=0.926 and R^2=0.848 were found for the IRGS and the IRG, respectively in the present study (Table 2, Figure 1f).

For the variables SC *vs* age, the correlation values were R=0.189 for the IRG and R=0.816 for the IRGS. In Menz lambs, the SC showed a high correlation with the age (R=0.83), although in this case it was also influenced by the nutritional level (Mukasa-Mugerwa and Ezaz, 1992). The results obtained confirm that when the ingestion of protein is increased above the requirements for maintenance and growth, puberty and fertility can be attained at an earlier age in small ruminants (Saab et al., 1997). In a study that included the Pelibuey breed, the SC and the age at puberty presented a correlation value of R=0.59 (Valencia et al., 2005). In the present study, values of R^2=0.664 and R^2=0.029 were founded for the IRGS and the IRG, respectively (Table 2).

As to hormonal activity, the values of testosterone for the IRGS group during the rainy season reached their maximum at 177 days of age (Figure 3) with 2.44±0.61 ng/ml, which coincides with the onset of histological puberty; this synchronization is comparable to finding an elevation in testosterone of 0.78 ng/ml at 32 weeks, coincident with puberty (Avellaneda et al., 2006) in different ovine breed. Both findings confirm the stipulation by Herrera-Alarcon et al. (2007), affirming that testosterone values may be used as possible indicators of puberty in ovine males.

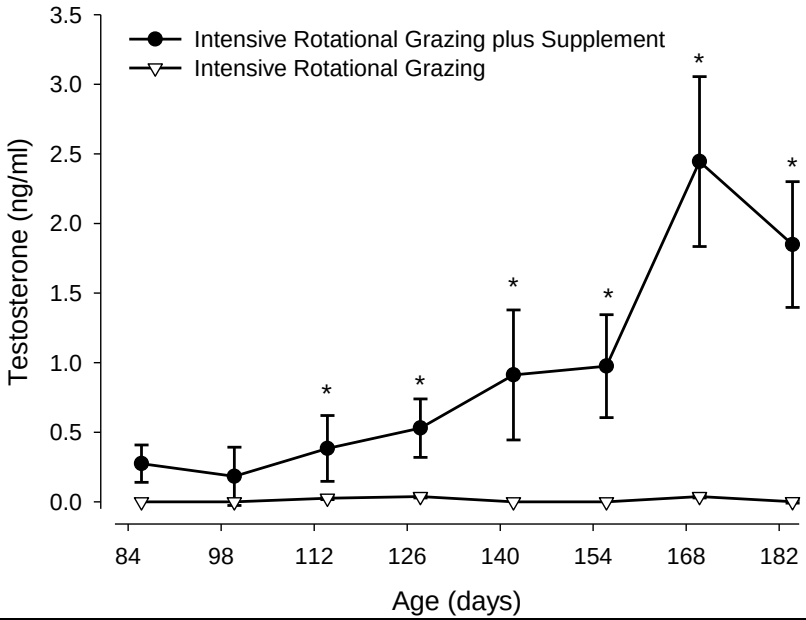

Figure 3. Testosterone concentrations (ng/ml) in serum of Pelibuey rams under conditions of Intensive Rotational Grazing plus Supplement (●) and Intensive Rotational Grazing (▽) *(P<0.05)

CONCLUSIONS AND IMPLICATIONS

Testicular development depends more on BW than on age, this being a reflection of the nutritional management under which the rams develop. Testicular measurements can be used as a tool for detecting animals that have been raised on nutritionally poor diets, although exists a considerable margin in the genetic plane that should be taken into account when making selections.

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PRELMINARY INVESTIGATION OF AFLATOXINS IN DIETARY RATION OF DAIRY COWS IN KHARTOUM STATE, SUDAN

W.O.M. ELTEIB[1], I.E.M. El ZUBEIR[1], A.M.A. FADEL ELSEED[2], A.A. MOHAMED[3]

[1]*Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Postal code 13314, Sudan*
[2]*Department of Animal Nutrition, Faculty of Animal Production, University of Khartoum, Postal code 13314, Sudan*
[3]*National Chemical Laboratory, Ministry of Health*

*E-mail: Ibtisammohamed@hotmail.com

ABSTRACT: *This is a preliminary investigation of the incidence and levels of aflatoxins in dairy cow ration in Khartoum North locality using HPLC. The survey was based on three level of groundnut cakes concentration (low=16-18, medium=19-24 and high=25-32%). The data indicated that 2 out of 18 samples examined were contaminated with aflatoxins B1 (0.013 and 0.014 ppb), these values were below the maximum acceptable limit for dairy cows feeds (20 ppb) as was stated by FAO (1997). However further examination of 2 samples of groundnut cakes from the farms showing the positive sample, revealed 108.3 and 18.4 ppb for B1 and 71.6 and 12.4 ppb for B2, respectively. The study also suggested a relationship between the levels of groundnut cakes level in the feed ration of the dairy cows and the contamination by aflatoxins, as these positive samples were from feed ration of high level of groundnut cakes concentration. The positive samples were from dairy farms that mixed their own ration using a traditional mill. The study also showed the absence of G1, G2 and B2 in dairy cows feeding in Khartoum North locality. From this study it was concluded that ration formulation with different feedstuff could minimized the aflatoxins health risk for dairy animals, however further research is needed in this field.*

ORIGINAL ARTICLE

Key words: *Aflatoxins, Groundnuts Cakes, Dairy Cows, Contamination*

INTRODUCTION

A major handicap facing wide use of groundnut is its high susceptibility to growth and development of moulds, therefore contamination with mycotoxins, notably aflatoxins. Aflatoxins is a group of toxic metabolites, produced by strains of *Aspergillus flavus* and *A. parasiticus*, which proved to be highly toxic to a wide range of animal species including dairy herd (El-Nazemi et al., 2002). Studies have been conducted on aflatoxins contamination of agricultural products in the Sudan (Ali, 2004; Omer et al., 2004). These studies revealed a wide range of aflatoxins content in groundnut seeds and cakes under variable cultural practices, processing techniques and storage conditions. Some of these products were found to be highly contaminated with aflatoxins to an extent, which would limit their use in human foods or animal feeds (Omer et al., 2004). Milk, eggs and meat products are sometimes contaminated because of the animal consumption of aflatoxins contaminated feed (Martins et al., 2007; Hainaut and Boyle, 2008).

Aflatoxins are a group of mycotoxins, which have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect (Ozay et al., 2008). Afaltoxins are classified into B1, B2, G1 and G2; which metabolized to aflatoxins M1 and M2 (Boudra et al., 2007). Aflatoxins B1 is a potent mutagenic and carcinogenic agent found in numerous agricultural and dairy products consumed by humans (Maridgal-Santillan et al., 2007). Aflatoxins contaminated corn and cotton seed meal in dairy rations have resulted aflatoxin M1 contaminated milk and milk products, including skimmed milk, cheese and yoghurt (Van Eijkeren et al., 2006) and pasteurized milk (Zinedine et al., 2007). This study is designed to declare the incidence of aflatoxins in dairy cattle containing ground nut cakes in Khartoum North.

MATERIALS AND METHODS

Source of samples

Eighteen samples of ration were collected randomly from Khartoum State. Samples collection depended on the percentage of groundnut cake in the ration (low: 16-18, medium: 19-24, and high: 25-32%) of dairy cows in

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some of the dairy farms. The dairy rations with positive samples were further investigated by examination of the groundnut cakes for the levels of aflatoxins.

Source of materials

Methanol, chloroform, acetone, iso-propanol and anhydrous sodium sulfate were all of analytical grade. Standard aflatoxins B1, B2, G1 and G2 were Sigma products.

Laboratory examination of ration sample

High Performance Liquid Chromatography (SHIMADZU, DGU-20A3, PUMP, LC-20AB, OVEN CTO-20AC, RF 10AXL, Fluoresce detector), which was made in Japan, 2005 was used for detection of aflatoxins. The extraction was done as was described in AOAC (1980).

Extraction of sample

Fifty grams of each sample were put in 500 ml blender jar to which 200 ml of methanol: water (80:20) v/v was added. The contents were vigorously mixed for 3 minutes (in high speed blender). The extract was filtered through 24 cm Whatmann No.4, in a glass funnel. The filtrate was collected into 250 ml conical flask and moved to separatory funnel. Then 50 ml of 10% sodium chloride (NaCl) was added into the separatory funnel and 50 ml n-hexane was also added to form slurry. After proper mixing, the mixture was separated and the lower aqueous layer was drained.

The obtained liquid was transferred into another 250 ml separation funnel and 50 ml chloroform was added to extract and drain the organic layer. The mixture was shaken gently for 1 minute after which 25 ml chloroform was also added in order to re-extract. The lower chloroform layer (extract) was collected into 250 ml volumetric flask through a layer of anhydrous sodium sulphate (15 g). The collected chloroform extract was evaporated to almost dryness in a water bath, using anti-bumping granules. This extract was kept in a conical flask at room temperature until used.

Column chromatography cleanup

Two grams of silica gel slurry was put in the chromatography column and then 30 ml ether: hexane (3:1) v/v was added to the wash column and silica gel. This was drained off through the stopcorek. The sides of the column were washed also with 2-3 ml of ether: hexane solvent. After that silica gel was settled fully to the open stopcorek and while it was drained, the granular anhydrous (Na_2SO_4) was added to the top of the column. The stopcorek was closed and 2 ml of the chloroform extract was poured into the column and the beaker was washed with 0.5 ml chloroform, which was added to the column. Following this was the addition of 25 ml benzene: acetic acid (9:1) v/v into 250 ml beaker. Ether: hexane (30 ml) was added, while the stopcorek was fully opened to wash the top of the anhydrous Na_2SO_4 layer. To the eluted aflatoxins, 100 ml of dichloromethane: acetone (90:10) v/v was added and the mixture was transferred to a boiling water bath for evaporation of the solvents. The concentrated toxin was transferred quantitatively to a vial using 0.5 ml pipette.

Derivatization

Derivatization of samples were performed by adding 200 µl hexane and 50 µl Trifluroacetic acid (TFA) were added to extract in vial column and capped. The mixture was shaken vigorously using vortex-Genie 2 for 30 seconds and left to stand for 5 minutes. Then 1.950 ml acetonitrile - water (1:9) v/v was added and the mixture was shaken for 30 seconds. The mixture was allowed to stand for 10 minutes to separate. The lower aqueous layer was collected by automatic pipette and used for High Performance Liquid Chromatography (HPLC). Similarly derivation of working standard mixture was done by taking 50 µl of standards and the solvent was evaporated and 200 µl of hexane and 50 µl Trifluroacetic acid (TFA) were added to column extract. Then 1.95 ml acetonitrile: water (1:9) was added and shacken to mix for 30 seconds. The layer was then separated for 10 minutes and the lower aqueous layer was used for HPLC analysis.

HPLC condition

Column length 20 µl, Fluorescent Detector: 360 nm excitation, 400 nm emission, flow rate= 1 ml/min, Oven Temperature = 20 °C, Injection Volume = 20 µl and Sensitivity= medium.

Liquid chromatography system

Samples were compared with standard peaks (Figure 1 and Figure 2), and the concentration in the samples was calculated by using either peak heights or area. Derivatization was performed because of aflatoxins B1 and G1 in aqueous solvents on chromatogram of standard mixture and samples. There were four peaks G2a (from G1), B2a (from B1), G2 and B2 with apparent retention times of 11, 15, 23 and 33, respectively.

Method of aflatoxins calculation

Aflatoxins concentrations were calculated in µg/kg from the following formula:

Concentration of aflatoxins in sample/ µg/kg:-

$$= \frac{\text{area of sample} \times \text{concentration of standard}}{\text{area of standard} \times \text{weight of sample}}$$

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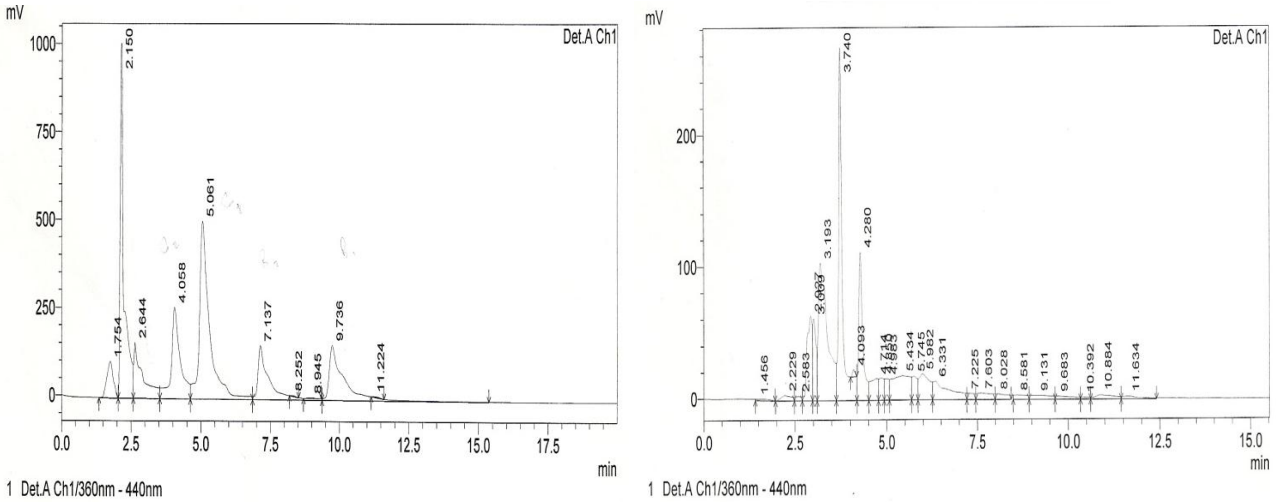
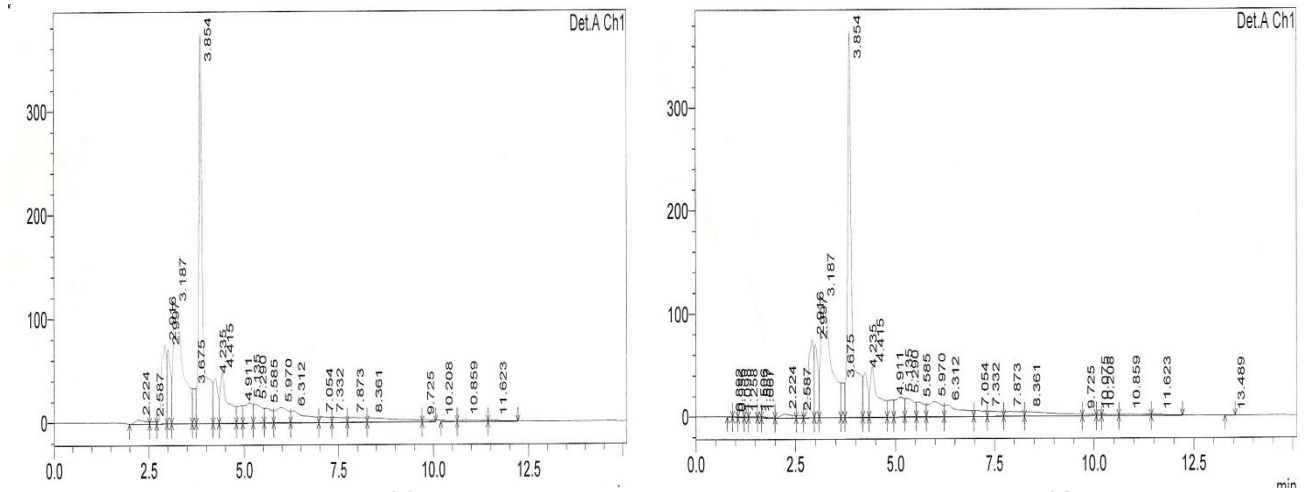
Where:
Concentration of (B1) in standards =0.50 µg/ml
Concentration of (B2) in standards =0.25 µg/ml
Concentration of (G1) in standards =0.50 µg/ml
Concentration of (G1) in standards =0.25 µg/ml



a. Standard b. negative sample

Figure 1 - Aflatoxin peaks of the standard and a negative sample (sample no. 1)



a. sample no. 11 b. sample no. 18

Figure 2 - Aflatoxin detection peak of a positive sample (sample no. 11 and 18)

RESULTS AND DISCUSSION

The present study was conducted in Khartoum North in Khartoum State. The main objective of this study was to evaluate the level and incidence of aflatoxins in the ration of dairy cows. This study showed that aflatoxins B1 was present in the feed of dairy animals, while aflatoxins G1, G2 and B2 were not detected (Table 1). The presence of aflatoxins B1 in the dairy cows ration might create a hazard because the effect of aflatoxins B1 is accumulation (Omer et al., 1998). A linear relationship between the cow's lactation status and feed intake, the daily milk production and aflatoxins B1 concentration in total feed related to aflatoxins M1 level in milk was demonstrated (Van Eijkeren et al., 2006). Moreover aflatoxins B1 is very hazardous to humans and animals as it was regarded as carcinogenic (Omer, 1998; Fardohan and Zoumenou, 2005; Surendranatha Reddy et al., 2011.). Attention should be directed towards the control of aflatoxins especially in cake in dairy cow ration. Many factors were found to affect significantly the incidence of aflatoxins contaminations in groundnuts cake such as type of soil, method of harvesting of crop, method of oil extraction, storage period and the type of store (Ali, 2004). Similarly the moisture content (Omer et al., 2004), the storage conditions (Stephen- Blezinger, 2002; Pazzi et al. 2005), low temperatures (Ghorbanian et al., 2008), relative humidity (Giorni et al., 2007), the season (Tajkarimi, et al., 2007), damaged pods (Ozay et al., 2008) are all been reported as factors. However good agricultural practices durig both pre and post harvest conditions would minimize the problem of contamination by aflatoxins (Stephen-Blezinger, 2002). These include appropriate drying techniques, maintaining proper storage facilities and taking care not to expose grains or oil seeds to moisture durig transport and marketing (Magan and Aldred, 2007).

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Table 1 - Incidence of aflatoxins in the ration of dairy cows in Khartoum North

Percentage of groundnut cake	Sample area	Percentage of groundnut cake in sample	Sample No	Aflatoxins µg/kg			
				G2	G1	B2	B1
Low 16-18%	U of K farm Shabmat	18	1	ND	ND	ND	ND
	U of S farm Hilat KuKu	18	5	ND	ND	ND	ND
	Al-Kadro 1	16	9	ND	ND	ND	ND
	Al-Kadro 2	16	13	ND	ND	ND	ND
	Al-Haj Yosif 1	16	15	ND	ND	ND	ND
	Al-Haj Yosif 2	16	16	ND	ND	ND	ND
Medium 19-24%	Al-Samrab	24	2	ND	ND	ND	ND
	Um doum 1	20	3	ND	ND	ND	ND
	Shambat	24	7	ND	ND	ND	ND
	Al-Droshab	24	8	ND	ND	ND	ND
	Um doum 2	19	10	ND	ND	ND	ND
	Al-ailafon 1	24	12	ND	ND	ND	ND
High 25-32%	Research center Hilat KuKu	25	4	ND	ND	ND	ND
	Al-ailafon 2	28	6	ND	ND	ND	ND
	al-Halfaya 1	32	11	ND	ND	ND	0.013
	Al-Sababi 1	32	17	ND	ND	ND	ND
	Al-Sababi 2	32	14	ND	ND	ND	ND
	al-Halfaya 2	32	18	ND	ND	ND	0.014

ND: Not Detected , Limit of detection 0.1µ/kg

Two out of eighteen samples of the examined feed ration that were analyzed contained aflatoxins B1 (sample 11 and sample 18 which were collected from al-Halfaya area). One of the two farms showing the positive aflatoxins contamination was found to mix their own feed ration using a mill (they store feed ingredients), while the other farm purchase a ready mixed feed ration from a feed mill outside the farm (The further investigation showed that they were from the same source).

The first restore sample No.11 was positive to aflatoxins B1 showed a retention-time of 9.725 and the contamination level of aflatoxins B1 was calculated as 0.014 µg /kg (Figure 1) according to the procedure described in the technical manual. The second positive sample No.18 showed also high retention-time of about 9.725 and when compared with standard it revealed a level of contamination by 0.013 µg/kg. Both positive samples contain percentage of groundnuts cake about 32% to total ration (high level of groundnuts cake). Although the levels were lower compared to the detection limit for feed (20 ppb) stated by FAO (1997), this result is still hazardous because toxin of the accumulated level of aflatoxins (Omer et al., 1998; Fardohan and Zoumenou, 2005). Further investigation, revealed that the original groundnut cakes of sample no. 11 showed 108.3 and 18.4 and sample no. 18 showed 71.6 and 12.4 ppb for B1, B2, respectively (Figure 3). Moreover the detection of aflatoxins B1 in 33.3% of the total feed samples with the high concentration of ground nut cakes (Table 1) indicated that standards and regulation should be adopted in order to minimize level of contamination, because aflatoxins B1 is reported as one of the most potent and potentially lethal metabolite which is well known as human carcinogen (Guzman de Pena, 2007). On the other hand because aflatoxins are very hazardous to animal and human health, young calves are especially susceptible to these toxic effects, which might be largely due to under development of the rumen (Stephen- Blezinger, 2002). It was found that the aflatoxin B1 is directly related with the aflatoxin M1 (Van Eijkeren et al., 2006), B1 in feeds for animal consumption represents a serious problem to human and animal health (Fardohan and Zoumenou, 2005; Van Eijkeren et al., 2006).

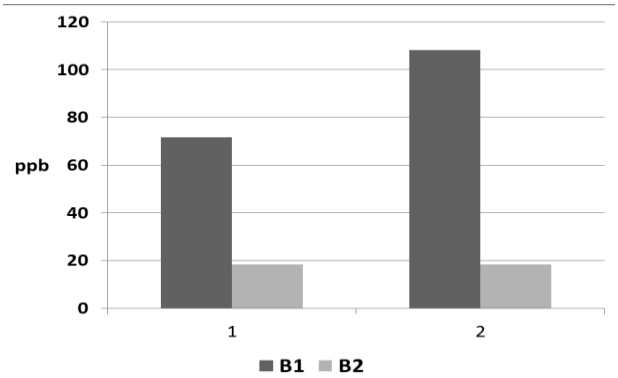

Figure 3 - Detection of Aflatoxin B1 and B2 in groundnut cakes from dairy cows' feed

Mycotoxins attract world-wide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (CTA, 1997). The economic impact of aflatoxins drive directly from crop and livestock losses as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health (Martins et al., 2007). Control measures include education on the risks of exposure to mycotoxins through skin contact, inhalation and ingestion, early harvesting, rapid appropriate drying, sequestration of diseased seeds from sound seeds, sanitation, use of good agronomic practices, insect control, the use of botanicals and synthetics as storage protectants, biological control and detoxification of mycotoxin-contaminated commodities (Negedu et al., 2011).

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The workshop hosted by World Health Organization to create an integrated plan intended to generate culturally appropriate, long-term, public health strategies to reduce aflatoxins exposure in developing countries (Hainaut and Boyle, 2008). The main recommendation stated clear strategy for afltoxin elimination. The present study support their recommendations include proper handling of crops to prevent mould infection and aflatoxins production in the field and examination or testing of groundnuts cake before addition to dairy cows feed. Also feed storage and distribution should be proper to eliminate the growth of fungus. Education and awareness should be implemented especially among farmers and livestock producers in addition to monitoring programs should be implemented and limits of aflatoxins should be stated for all food and feed. The level of groundnuts cake in dairy cow feeding should not exceed 18%.

The present study concluded that the presence of aflatoxins B in dairy animal feed especially in the high level of groundnut cakes might represents a serious problem of public health in both livestock and animals.

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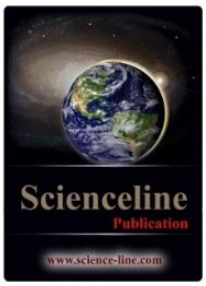

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		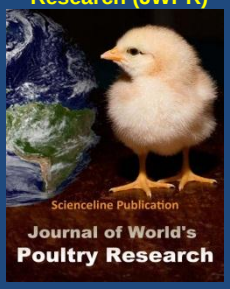	
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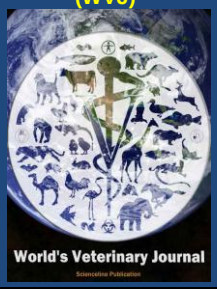		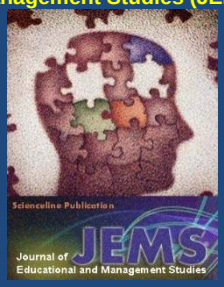	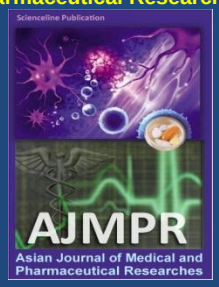
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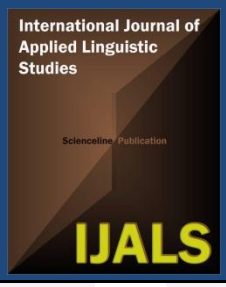			

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