



ISSN 2228-7701

Online Journal of Animal and Feed Research



An International Peer-Reviewed Journal which Publishes in Electronic Format



Online J. Anim. Feed Res., Volume 3, Issue 4: pp. 153-196; July 2013

Editorial team of OJAFR:

Administrator

Saeid Chekani Azar,
Dep. Anim. Sci., Facult. Vet. Med., Atatürk Univ., Erzurum, **TURKEY**

Managing Editor

Alireza Lotfi,
Dep. Anim. Sci., Islamic Azad University, Shabestar, **IRAN**

Editor-in-Chief

Habib Aghdam Shahryar,
Dep. Anim. Sci., Islamic Azad University, Shabestar, **IRAN**
(Assistant Prof., Nutrition - Non Ruminants)

Executive Editor

Mehrdad Ehsani-Zad,
MA in TEFL, Islamic Azad University, Takestan Branch, Takestan, **IRAN**

Editorial Board (A-Z)

Section Editors (SE)

Ahmad Yildiz,
Dep. Animal Science and Production, Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**
Ph.D., Associate Prof., **Nutrition - Ruminants**

Akbar Taghizadeh
Dep. Anim. Sci., Tabriz University, Tabriz, **IRAN**
Ph.D. Associate Prof., **Nutrition - Ruminants**

Ali Halajian
Dep. Biodiversity, School of Molecular and Life Sciences, Faculty of Science and Agriculture, University of Limpopo, **SOUTH AFRICA**
Ph.D. D.V.M., Professor of **Parasitology**

Ali Nobakht
Dep. Anim. Sci., I.A.U., Maragheh, **IRAN**
Ph.D., Assistant Prof., **Nutrition - Non-Ruminants**

Alireza Ahmadzadeh,
Dep. Anim. Sci., I.A.U., Shabestar, **IRAN**
Ph.D., Assistant Prof., **Biometry - Plant Breeding (Biotechnology)**

Alireza Lotfi,
Dep. Anim. Sci., I.A.U., Shabestar, **IRAN**
Physiology, Food Science and Technology

Ekrem LAÇIN,
Dep. Animal Science and Production, Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**
Ph.D., Associate Prof., **Nutrition - Non-Ruminants**

Fikret Çelebi
Dep. Physiology, Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**
Ph.D., Prof., **Physiology and Functional Biology of Systems**

Isaac Karimi
Department of Basic Sciences, School of Veterinary Medicine, Razi University, **IRAN**
DVM, DVSc, Assistant Prof., **Veterinary Physiology**

Hamid Mohammadzadeh
Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, **IRAN**
Ph.D., Assistant Prof., **Nutrition - Ruminants, Silage and silage additives, Carbohydrate fermentation, Microbial diversity in rumen and feces, Non-forage fiber sources, By-products**

Manish Kumar
Society of Education (SOE), India
Prof. Dr. **Pharmacology, Ethnomedicine**

Hamid Reza Gheisari
Academic staff, Dep. Food Hygiene, School of Vet. Med., Shiraz Univ., Shiraz, **IRAN**
Ph.D., Assistant Prof., **Biostatistics, Vet. Epidemiology, Food microbiology, Food chemistry and Meat Science. Dairy Science**

Hazim Jabbar Al-Daraji,
University of Baghdad, College of Agriculture, Abu-Ghraib, Baghdad, **IRAQ**
PhD, Professor of **Avian Reproduction and Physiology**

John Cassius Moreki
Ph.D., Department of Animal Science and Production, Botswana College of Agriculture, Gaborone, **BOTSWANA**
Nutrition - Non-Ruminants, Breeders, Nutritive value and utilization of feeds, Livestock management

Mohammed Yousof Kurtu
Animal Sciences Department, Haramaya University, Dire-Dawa, **ETHIOPIA**

Associate Prof., **Animal Science, Nutrition**

Khalid Mohammed Elamin Osman

Department of Animal breeding, Faculty of Animal Production, University of Gezira,

Ph.D., Assistant Prof., **Non-Ruminants, Genetics and Animal breeding, Mathematical models, analytical and experimental methods of feed evaluation, Animal-feed interactions.**

Naser Maheri Sis,

Dep. Anim. Sci., I.A.U., Shabestar, **IRAN**

Ph.D., Assistant Prof., **Nutrition - Ruminants, Nutritive Value, Utilization of Feeds**

Nilüfer SABUNCUOĞLU ÇOBAN,

Dep. Animal Science and Production, Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**

Ph.D., Associate Prof., **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**

Ph.D., Associate Prof., **Nutrition - Ruminants**

Paola Roncada

Department of Veterinary Medical Sciences, Facult. of Veterinary Medicine, University of Bologna, **ITALY**

Ph.D., Associate Prof., **Veterinary Pharmacology and Toxicology, Pharmacokinetics, Residues of mycotoxins in feed, in food and in food producing species, Residue depletion studies**

Saeid Chekani Azar,

Dep. Anim. Sci., Facult. Vet. Med., Atatürk University, Erzurum, **TURKEY**

Dep. Anim. Sci., Islamic Azad University (I.A.U.), Shabestar, **IRAN**

Product Quality, Physiology, Human Health and Well-Being,

Shahin Eghbal-Saeid,

Dep. Anim. Sci., I.A.U., Khorasgan (Isfahan), **IRAN**

Ph.D., Associate Prof., **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**

Dietary input, Animal and Feed interactions

Valiollah Palangi,

Dep. Anim. Sci., Islamic Azad University (I.A.U.), Maragheh, **IRAN**

Nutrition-Ruminants

Wafaa Abd El-Ghany Abd El-Ghany,

Poultry and Rabbit Diseases Department, Faculty of Veterinary Medicine, Cairo University, Giza, **EGYPT**

PhD, Associate Prof., Poultry and Rabbit Diseases

Zohreh Yousefi

Faculty of Biological Sciences, Shahid Beheshti University, Tehran, **IRAN**

Biology, Botanical Biosystematic (MSc), Plant Genetic (PhD student)

Wesley Lyeverton Correia Ribeiro

Medicine, State University of Ceará, Av. Paranjana, 1700, Fortaleza, **BRAZIL**

MSc, DVM, **Animal Health, Veterinary Parasitology, and Public Health, Animal welfare and Behavior, College of Veterinary**

Deputy Section Editors (DSE, Reviewers)

Arda Yildirim

Department of Animal Science, Faculty of Agriculture, Gaziosmanpasa University, 60240 Tokatö **TURKEY**

Ph.D., Assistant Prof., **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**

Nutrition-non Ruminants, Applied particles of Nanosilver in poultry production, Additives, Immune system, Nutrient digestibility

Ferdous 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

Animal Production Department, Faculty of Agricultural Sciences, Dalanj University,

Animal Science, Nutrition-non Ruminants, Nutritive Value, Utilization of Feeds

Mutaz Saeed Babiker Mahmoud

Dep. Poult. Prod., Facult. Anim. Prod., University of Gezira,

Non Ruminants

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,

Ph.D., Assistant Prof., **Animal-feed interactions, Nutritive value and utilization of feeds**

Peter Asiedu

Council for Scientific and Industrial Research (CSIR), Animal Research Institute (ARI), Box AH 20, Achimota-Accra, **GHANA**

M.Phil., **Animal Nutrition and Management**

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, **UNITED KINGDOM**

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



Friesian- Holstein rumen-cannulated steers (440±20kg) live weight. The objective of this study was to evaluate the usefulness of the browse legumes using the nylon bag technique. Nylon bags with 3g samples of dried ground legumes (3mm screen) were incubated in the rumen. The incubation times were 0, 6, 12, 48, 72 and 120 hours in four cannulated Friesian- Holstein steers. The browse legumes were analysed for nutritive value in terms of dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), Ash, condensed tannin (CT), calcium (Ca²⁺) and Phosphorus (P). Dry matter degradability was significantly different (P<0.05) and *Gliricidia* was highest, followed by *L. pallida* then *A. angustissima* and *C. calothyrsus* in descending order. Crude protein degradability was significantly (P<0.05) lower than that of DM and was highest in *L. pallida*, *G. sepium*, *A. angustissima* and finally *C. calothyrsus* at the bottom. Effective degradability of DM in the rumen of the steers was highest with *G. sepium* (880g/kg DM at rumen outflow rate of 0.02/h) and least with *C. calothyrsus* (504g/kg DM) (P<0.001). Effective degradability of nitrogen was highest with *L. pallida* (645g/kg DM at outflow rate of 0.02/h) and least with *C. calothyrsus* (103g/kg DM) (P<0.001). The degradability profiles of these browse indicated that they can be used as alternative protein supplements.

Key words: Nylon Bag Technique, Degradability, Effective Degradability, Ruminant, Rumen Cannulated Steers, Rumen Outflow Rates

Watch Online

Correlations among concentrations of some metabolic hormones and nutritionally-related metabolites in beef cows



Original Research, C34
Dampney J.K., Obese F.Y., Aboagye G.S., Ayizanga R.A.
Online J. Anim. Feed Res., 3(4): 176-180, 2013.

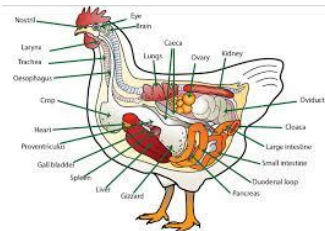
ABSTRACT: A study was conducted to investigate correlations among some metabolic hormones and nutritionally-related metabolites in plasma samples from sixteen multiparous Sanga cows raised extensively on natural pasture during early lactation. Blood was sampled from cows once every two weeks, from week 1 to 9 postpartum. The samples were processed for plasma and concentrations of the metabolic hormones and nutritionally-related metabolites were measured. Insulin-like growth factor-I (IGF-I) was positively correlated with insulin (0.377; P<0.001) and glucose (0.249; P<0.05), but negatively correlated with urea (-0.241; P<0.05). Insulin was positively correlated with glucose (0.440; P<0.05), total protein (0.262; P<0.05), and albumin (0.242; P<0.05), but negatively related with cholesterol (-0.279; P<0.05). Leptin was correlated positively with total protein (0.338; P<0.001) and albumin (0.351; P<0.001). There was a positive correlation between glucose and total protein (0.410; P<0.001) or albumin (0.425; P<0.001), but the correlation with urea was negative (-0.291; P<0.01). Total protein was positively correlated with albumin (0.682; P<0.001), but negatively correlated with cholesterol (-0.561; P<0.01). Furthermore, albumin was negatively correlated with creatinine (-0.294; P<0.01), while cholesterol was positively correlated with urea (0.253; P<0.05), and creatinine (0.294; P<0.01). The positive relationships among the nutritionally-related metabolites and metabolic hormones suggests that the effect of alterations in energy balance and (or) protein balance on postpartum ovarian function could be mediated through changes in the secretory patterns of these metabolic hormones.

Key words: Body Condition Score, IGF-I, Ovarian Activity, Postpartum Period



Watch Online

Effect of surgical removal of the residual yolk sac on the development of the digestive system and immune response in broiler chicks during early days post-hatch



Original Research, C35
Osama. H. A. Ali and Huwaida E.E. Malik
Online J. Anim. Feed Res., 3(4): 181-185, 2013.

ABSTRACT: This study was designed to investigate the effects of the residual yolk-sac on the development of the digestive system and immune response of broiler chicks. Two experiments were conducted in this study. In the first one, 60 day-old broiler chicks (Lohmann) were allocated into three experimental groups according to the status of the residual yolk sac; deutectomized (surgically-removed residual yolk sac), sham operated and intact chicks. Five chicks from each experimental groups were randomly selected at days 2, 4, 6 and 8, weighed, euthanized and the different parts of their digestive tract and liver were weighed. The body weight of deutectomized chicks at day 2, 4 and 6 post-hatch was significantly (P < 0.05) lower compared to that of sham operated and intact chicks. At day 8, the body weights of all experimental groups did not significantly (P < 0.05) differ from each other. The liver weight in deutectomized chicks was significantly (P < 0.05) lower at days 2, 4 and 6 post-hatch compared to that of the other experimental groups. At day 8 the liver weights in the different experimental groups did not show any significant difference. The weights of the different parts of the digestive tract (crop, gizzard, proventriculus, intestine), somehow, in deutectomized chicks were significantly (P < 0.05) lower at day 2, 4 and 6 compared to that of sham operated and intact ones. In the second experiment, 60 day-old broiler chicks (Lohmann) were allocated to the above mentioned experimental groups; 20 chicks per each. Thereafter, they were challenged with 10% sheep RBC suspension at day 2 and day 12 post-hatch. Ten chicks were randomly selected from each experimental group at day 12 and day 20 post-hatching, killed and their lymphoid organs (spleen, thymus and bursa of fabricius) were incised and sera were harvested from blood samples. The lymphoid organs were significantly (P<0.05) lower in deutectomized chicks compared to the two other experimental groups. The geometric mean titers (GMT) of antibodies against 10% sheep RBC suspension for primary and secondary immune responses in deutectomized chicks, were lower than that of sham operated and intact chicks. The results of this study revealed that the residual yolk sac is essential for the development of the digestive system and immune response in broiler chicks.

Key words: Broiler Chicks, Deutectomy, Digestive system, lymphoid organs, Immune response.



Watch Online

Pathogenic microorganisms isolated from periwinkles in creeks south-south of Nigeria



Original Research, C36

Nwiyi P., Okonkwo C.

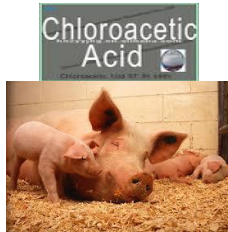
Online J. Anim. Feed Res., 3(4): 186-188, 2013.

ABSTRACT: One hundred and twenty pieces of periwinkle were obtained each from Yenogoa and Oron Creek. The periwinkles were of two genera namely: *Pachymelania aurita* obtained from Oronk Creek located in Akwa-Ibom State, while the *Tympanotonus fuscatus* notably a brackish water habitat was obtained from Yenogoa in Bayelsa state both in south-south Nigeria. Evaluation of possible microbiological isolate was carried out according to Cowan and Steel's Manual for medical Bacterial identification. The Creek in Yenogoa presented high level of Coliform count $2.6 \times 10^5 \text{cfug}^{-1}$ while the Oron Creek had an unacceptable load of *Salmonella* count $6 \times 10^6 \text{cfug}^{-1}$. The total bacterial count was highest in Oron Creek $1.46 \times 10^8 \text{cfug}^{-1}$ from *Tympanotonus fuscatus*. The microorganisms isolated from both Creeks were *Escherichia coli*, *proteus sp*, *salmonella sp*, *pseudomonas sp* and *Enterobacter sp*. *Proteus sp* was the least isolated while *Salmonella sp* was the highest. **Key words:** Pathogenic microorganisms, periwinkles, South-South, Nigeria.



[Watch Online](#)

Growth performance and haematological parameters of weanling pigs fed diets supplemented with chloroacetic acid



Original Research, C37

Amaechi N. and Njoku U.P.

Online J. Anim. Feed Res., 3(4): 189-192, 2013.

ABSTRACT: This study investigated the effect of chloroacetic acid on growth performance and haematological parameters of weanling pigs. Thirty-six cross-bred weanling pigs (Landrace X Duroc) were allotted randomly to four treatment groups, with three replicates of three weanling pigs in each group. Control (T_1) weanling pigs were given a standard basal diet; Treatment 2, 3 and 4 were diets of 0.3, 0.6 and 0.9 percents levels of inclusion of chloroacetic acid respectively. After six weeks, blood and intestinal samples were collected from one animal per replicate. Data on feed intake and weight gain were collected daily. Results showed that chloroacetic acid did improve the animal growth performance. There was a decrease in pH. There were significant differences ($P < 0.05$) on white blood cell and mean corpuscular haemoglobin across the treatment. There was no significant difference ($P < 0.05$) across the treatments on pack cell volume and red blood cell count. This study showed that chloroacetic acid influenced some haematological parameters, decreased the pH of the gastro-intestinal tract of the animals. Further studies will be needed to better understand the mechanisms underlying the effects observed when chloroacetic acid is fed to weanling pigs. **Key words:** Nitrogen Chloroacetic Acid, Growth Performance, Haematological Parameters Weanling Pigs.



[Watch Online](#)

Effect of dietary levels of spearmint (mentha spicata) on broiler chicks performance



Original Research, C38

Amasaib E.O., Abd Elrahman B.H., Abdelhameed A.A., ATTA ELMNAN B.A. and Mahala A.G.,

Online J. Anim. Feed Res., 3(4): 193-196, 2013

ABSTRACT: This study was conducted to determine the effect of addition different levels of spearmint (*Mentha spicata*) on broiler chick's performance. One hundred and twenty eight day old unsexed (Cobb) broiler chicks were used in this experiment. Birds were distributed randomly into 16 pens (8/pen) as replicates, in a complete randomized design. The experimental diets were formulated with four levels of spearmint (*Mentha spicata*) of 0, 1, 1.5 and 2%. Feed and water were freely accessed. Feed intake, body weight gain and feed conversion ratio were weekly recorded and Mortality rate was recorded throughout the experiment. At the end of the experimental period, four birds from each treatment were randomly selected, weighed and slaughtered for determination of carcass weight and dressing percentage. Average feed intakes obtained from the experiment were 2680.20, 2679.11, 2708.55 and 2692.57 for diets 0, 1%, 1.5% and 2%, respectively. However, the body weight gain for the treatments were 1481.63, 1512.81, 1519.57 and 1519.63, 0, 1%, 1.5% and 2%, respectively. Feed conversion ratios for treatments were found to be 1.92, 1.94, 1.92 and 1.99 respectively. Dressing percentage were 73.12, 74.17, 73.08 and 73.47 respectively. The results indicated that the supplementation of different levels of spearmint to the diets of broiler improved feed intake and body weight gain. **Key words:** Spearmint (*Menthaspicata*), Broiler, Performance



[Watch Online](#)

✂ Join OJAFR Team

Online Journal of Animal and Feed Research (OJAFR) is published in Iran. As an international journal we are always striving to add diversity to our editorial board and operations staff. Applicants who have previous experience relevant to the position they are applying for may be considered for more senior positions (Section Editor, SE) within OJAFR. All other members must begin as Deputy Section Editors (DSE) before progressing on to more senior roles. Editor and editorial board members do not receive any remuneration. These positions are voluntary.

If you are currently an undergraduate, M.Sc. or Ph.D. student at university and interested in working for OJAFR, please fill out the application form below. Once your filled application form is submitted, the board will review your credentials and notify you within a week of an opportunity to membership in editorial board.

If you are Ph.D., assistant, associate editors, distinguished professor, scholars or publisher of a reputed university, please rank the mentioned positions in order of your preference. Please send us a copy of your resume (CV) or your Live DNA or briefly discuss any leadership positions and other experiences you have had that are relevant to applied Animal and Feed Researches or publications. This includes courses you have taken, editing, publishing, web design, layout design, and event planning.

If you would like to represent the OJAFR at your university, join our volunteer staff today! OJAFR representatives assist students at their university to submit their work to the OJAFR.

You can also, registered as a member of OJAFR for subsequent contacts by email and or invitation for a honorary reviewing articles.

Contact us at editors@ojafr.ir or editorojafr@gmail.com

Download [OJAFR Application Form](#):



✂ Contact Information

Please contact

For your questions or comments about OJAFR with administrator

By Email: schekani@gmail.com

.

For submission of your work, cooperating and recommendations with OJAFR's managing editor

By Email: editors@ojafr.ir

.

For Editorial and Author Enquiries

Editor-in-Chief (Email):

h_a_shahryar@yahoo.com

Editorial Boards (Email):

editors@ojafr.ir



Scienceline Publication

In Turkey (Office): Atatürk University, Yakutiye/ Aziziye, P.O.BOX 25010/25100, Erzurum City/Province, TURKEY

In Iran: PO BOX 551 (Goddusi Street), Maragheh, East Azerbaijan Province, IRAN

Telephone: Turkey: +905387708824, Iran: +989144207713

Fax: +90 421 222 3950

Email: administrator@science-line.com , scil.publishing@gmail.com

PERFORMANCES OF CATLA (*Catla catla*) FINGERLING REARED ON LOCALLY AVAILABLE FEED INGREDIENTS

P. P. SRIVASTAVA*, J. K. JENA, S. CHOWDHARY, P. SHARMA, S. RAIZADA and R. DAYAL

National Bureau of Fish Genetic Resources, Canal Ring Road, Telibagh, Lucknow – 226002, UP, India

*Email: ppsicar@gmail.com

ABSTRACT: A comprehensive trial was undertaken to assess the effect of various types of feed ingredients on the biomass conversion rate in a 12-week feeding trials to evaluate the use of agro-based products, as locally available feed ingredient materials for fish catla (*Catla catla*) fingerling (av. wt. 1.52±0.11 to 1.55±0.07 g) growth performances. In experiment, three (26.14 to 26.56 % crude protein) practical diets were formulated. The experimental diets were fed to five replicate groups of fingerlings at 8% of body weight and results were compared. After 12-week study the final weight gain recorded as 12.45±0.03 g, 15.23±0.15 g and 18.12±0.17 g in F1 to F3 fishes respectively. The percentage weight gain recorded as 719.1%, 895.4% and 1069.0% respectively from initial weight. The results suggest that the growth is better in feed F3 containing higher soybean meal, Potato starch and lower content of Mustard oil cake. The feed conversion ratio (FCR) ranged between 2.34±0.11 to 2.98±0.09. The survival was recorded in F1 to F3 as 60±4.1%, 70±2.3% and 80±3.3% respectively. Lipid and protein contents in carcass composition differ significantly (P<0.05) among the three feeding trials. The study suggests that the soybean diet which is more effective, than the mustard oil cake, in the deposition of nutrients in terms of flesh (at early growing stage of life), and led to be significantly higher (P<0.05) growth than the other two diets in, *Catla catla*.

Key words: Feed ingredients, Fingerling, Growth performances, *Catla catla*

INTRODUCTION

The Indian major carp, *Catla catla*, is a promising species for aquaculture exploitation with its rapid growth and good market potential. In terms of value-added, processed fish products, this species should have potential as the present market price of this fish is ranging between Rs. 80 to 140 per kilogram in Indian markets. One key biological component is the availability of suitable diets that are efficiently digested and supplement the required nutrients for optimizing growth performances (Mokolensang et al., 2003). The suitability of using formulated feed sources will provide cost effective management practices for efficient fish growth with cheaper feed ingredient under culture system in this herbivore fish, Indian major carp (*Catla catla*) for aquaculture candidate species. Indian major carp, *Catla catla*, is a valuable herbivorous food fish in India. Polyculture of Indian major carps (IMC) viz: Rohu (*Labeo rohita*), Catla (*Catla catla*) and Mrigala (*Cirrhinus mrigala*) is mainly dependent on plant-based agro-by-products. All three species of IMC (Rohu, Catla and Mrigala) are known to be capable of utilizing dietary protein and carbohydrates well, with even cellulolytic activity in Rohu (*Labeo rohita*) as described by Saha and Ray (1998) and Das and Tripathi (1991). Sabapathy and Teo (1993) have reported amyolytic activity in major carp. Rohu are able to utilize complex polysaccharides more efficiently than simple sugars (Erfanullah and Jafri, 1995, 1998). Protein sparing effect of carbohydrate in fry and fingerlings of rohu, mrigal and common carp was demonstrated by Rao (1987). There was no weight gain difference recorded on fry fed diets with 40:30 and 45:25 w/w protein: carbohydrate levels and protein sparing effects have been recorded (Sen et al., 1978). Supplementary feeding is known to increase the carrying capacity of culture systems and can enhance fish production by many folds (Devaraj et al., 1976). It also offers the best means of fish production within the shortest possible time in the ponds.

Attempts have been made to understand the gross level of nutrient requirements for proteins, lipids, carbohydrates, vitamins and minerals for Indian major carps (De-Silva and Gunasekera, 1991; Balogu et al., 1993; Saeed et al., 2005). The role of artificial feed in fish farming cannot be avoided as nutritional requirements of fish depend upon the feed supplied. The quantity and quality of feed consumed have a impactful effect on growth, feed conversion and proximate composition of fish (Hassan et al., 1996; Jena et al., 1998; Erfanullah and Jafri, 1998). The FCR values of various fish feeding ingredients for carps under controlled conditions have been studied by many researchers (Jhingran, 1991; Shabbir et al., 2003; Jabeen et al., 2004; Ali and Salim, 2004; Saeed et al., 2005; Inayat and Salim, 2005; Gull et al., 2005). Thus this study was planned to observe the growth of catla on feeding with specified agro-based by-product sources and this study will be much useful to determine the role of supplementary nutrition for weight gain and survival rate of the *Catla catla* fingerling.

MATERIALS AND METHODS

ORIGINAL ARTICLE



The hatchery-bred spawn of catla, after acclimatization, were fed with laboratory made egg-custard feed (Table 1). The healthy fish were separated to conduct feeding experiment.

Nutritional Studies on fingerling: Feed preparation and feeding

During the acclimation the fish were fed *ad libitum* with the moist feed containing Mustard oil cake, Wheat flour, Soybean meal and vitamin and mineral mix mixed in a ratio of 30:20:48:2 w/w (Table 2) for further weaning and rearing on artificial feed. After seven days various economical feeds with gross protein as 26.14 – 26.56% (Table 3) were formulated and growth study was carried out for 12 week rearing period for the fingerling of *C. catla* with different feeds and the growth performances was recorded (Table 4).

Table 1 - Feed compositions used during rearing of *C. catla* spawn

Ingredients	Percentage
Hen egg white	28.0
Lactogen powder	60.0
Fishmeal powder	10.0
Vitamin & Mineral Mix*	2.0

*From 'Agrimin Forte' contains Vit. A 700000 IU, Vit. D₃ 70000 IU, Vit. E 250mg, Nicotinamide 1000mg, Co 150mg, Cu 1200mg, I 325mg, Fe 1500mg, Mg 6000mg, Mn 1500mg, K 100mg, Se 10mg, Na 5.9mg, S 0.72%, Zn 9600mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, Doddaballpur Ind. Area, Doddaballapur - 561 203, India Batch No. BFA-61.

Table 2 - Feed compositions used during acclimatization of *C. catla* fry

Ingredients	Percentage
Mustard oil cake	30.0
Wheat Flour	20.0
Soybean meal	48.0
Vitamin and Mineral Mix*	2.0

*From 'Agrimin Forte' contains Vit. A 700000 IU, Vit. D₃ 70000 IU, Vit. E 250mg, Nicotinamide 1000mg, Co 150mg, Cu 1200mg, I 325mg, Fe 1500mg, Mg 6000mg, Mn 1500mg, K 100mg, Se 10mg, Na 5.9mg, S 0.72%, Zn 9600mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, Doddaballpur Ind. Area, Doddaballapur - 561 203, India Batch No. BFA-61.

Table 3 - Feed compositions used during rearing of *Catla catla* fingerling

Feed	Mustard Oil Cake (%)	Potato Starch (%)	Rice Polish (%)	Soybean meal (%)	Vitamin Mineral* (%)	Gross protein (%)
F 1	43	8	31	17	1	26.14
F 2	32	5	40	22	1	26.14
F 3	19	9	40	31	1	26.56

*From 'Agrimin Forte' contains Vit. A 700000 IU, Vit. D₃ 70000 IU, Vit. E 250mg, Nicotinamide 1000mg, Co 150mg, Cu 1200mg, I 325mg, Fe 1500mg, Mg 6000mg, Mn 1500mg, K 100mg, Se 10mg, Na 5.9mg, S 0.72%, Zn 9600mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, Doddaballpur Ind. Area, Doddaballapur - 561 203, India Batch No. BFA-61.

Protein contents in ingredients and feed:

Protein** contents in feed and ingredients are given below -

Protein in mustard cake (MOC) was	= 32.0 %
Potato Starch	= 2.0 %
Rice Polish	= 12.0 %
Soybean meal	= 50.0 %

Footnote: Average protein contents in prepared feed ranged between 26.14 – 26.56% from F1 to F3.(Feed 1

Crude Protein, 26.14 %; Feed 2 Crude Protein, 26.14 %; Feed 3 Crude Protein, 26.56%.

** Protein estimated using N x 6.25

Physico-chemical parameters of water

The water quality of rearing tanks was analysed following the standard methods (APHA 1998) and was found in normal range with temperature 26±1 OC, pH 6.5-7.1, total alkalinity 129-136 ppm and dissolved oxygen 6.8-7.3 ppm.

Analytical methods and analysis of data

Growth Performance Activities: The fingerling of catla were kept in separate tanks/pools with five replicates per feed totalling fifteen pools and were fed *ad libitum* with different feeds in these fifteen pools (300 l capacity) arranged in Random Block Designing. The performance of the feeds, in terms of the weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), Protein efficiency ratio (PER). The growth in length and weight and the survival data were analysed using one-way ANOVA. Duncan's multiple range test was used to determine which treatment means differed significantly (P<0.05) using SPSS version 16.0.

The weight gain, specific growth rate, survival and biomass were calculated using the following formulae.

Weight Gain (%) = {(Final body weight) – (Initial body weight)/ (Initial body weight)} x 100



Specific Growth Rate (SGR; % day⁻¹) = ((Final body weight) - (Initial body weight) / (experimental days))x 100

Survival (%) = 100 x (No. of total fish - No. of dead fish)/Number of total fish

Biomass = Final average weight x Total no. of fish

The results were recorded in terms of specific growth (SGR), protein efficiency ratio (PER), per day increment (PI) and feed conversion ratio/efficiency (FCR) (Table 4 & 6). The survival was recorded at the end of the 4th, 8th and 12th week (Table 4 and 7).

Biochemical Analysis

Proximate compositions of feeds and fish carcasses were analyzed in triplicate. Dry matter was estimated after drying in oven at 105 °C for 24 hours; crude protein (N x 6.25) by the Kjeldahl method) after acid digestion; crude lipid by di-ethyl ether extraction method using Soxhlet apparatus. Proximate analysis study was carried out for the reared fingerling of *C. catla*, fed with different feeds was analysed for body composition (Table -8). The body tissue, feed of the experiments were analysed for dry matter (DM), crude protein (CP), lipid and total ash according to AOAC (1990). The organic matter (OM) was calculated by subtracting the total ash from dry matter (DM).

RESULTS

The growth performances, survival and proximate composition of *C. catla* are depicted in Table -4, 5, 6, 7 and 8. The final weight gain, after 12th week, ranged between 12.45±0.03 to 18.12±0.17 g in F1 to F3 feeding trials. Growth parameters of *Catla* fingerling with different feed clearly showed significant enhancement with Mustard oil cake, potato starch, rice polish and soybean meal when compared with other concentrations of these four feed ingredients.

Catla fingerling showed maximum increase in length (45 mm), weight gain (16.57 g) were observed in F3 and similar trends were observed with F2 and F1 feeds.

The biochemical parameters of *Catla catla* fingerling fed with different types of food showed most favorable enhancement in the levels of proteins, lipids, FCR and SGR in F3, F2 and F1 feeds. *Catla* fingerling fed with F3 feed, the FCR, SGR, PI and survival were 2.34±0.11, 19.73, 215.7 mg and 80±3.3% respectively. Similarly the results of F1 and F2 were also encouraging.

Table 4 - The growth performance of the fingerling of *Catla catla*

Feed	Initial weight (g)	Final weight (g) 4 th week	Final weight (g) 8 th week	Final weight (g) 12 th week	Specific growth rate (SGR) after 12 weeks	Survival (%)	FCR
F-1	1.52±0.11 ^a	3.23±0.2 ^a	6.34±0.23 ^a	12.45±0.03 ^a	13.01 ^a	60 ±4.1 ^a	2.98±0.09 ^b
F-2	1.53±0.09 ^a	3.61±0.3 ^{c*}	7.76±0.21 ^b	15.23±0.15 ^b	16.31 ^b	70±2.3 ^b	2.55±0.10 ^a
F-3	1.55±0.07 ^a	3.84±0.1 ^b	8.87±0.12 ^b	18.12±0.17 ^c	19.73 ^c	80±3.3 ^{c,**}	2.34±0.11 ^{a,**}

Same alphabet in superscript in a column represents no significant difference in weight gain. * = p< 0.01 ; ** = p< 0.05. The results are of five replicates of feeding trial.

Table 5 – Initial and final weights and lengths, weight gain and percent weight gain of the *Catla catla* fingerling of different treatments during 12 week experimental period

Feed	In length (mm)	Fn length (mm)	In weight (g)	Fn weight (g)	length gain (mm)	% Length gain	Weight gain (g)	% Weight gain
F1	50±1 ^a	92±2 ^a	1.52± 0.11 ^a	12.45±0.03 ^a	42.0 ^a	84.0 ^a	10.93 ^a	719.1 ^a
F2	52±1 ^a	94±1 ^a	1.53± 0.09 ^a	15.23±0.15 ^b	42.0 ^a	80.8 ^b	13.7 ^b	895.4 ^b
F3	51±2 ^a	96±2 ^a	1.55± 0.07 ^a	18.12±0.17 ^c	45.0 ^b	88.2 ^c	16.57 ^c	1069.0 ^c

Means in a given column having the same letter superscript are not significantly different at (p <0.05) by ANOVA and Duncan multiple range test.

Table 6 – Average initial and final weight, protein efficiency ratio (PER) and per day increment (PI) of *C. catla* fingerling fed various experimental diets for 12 weeks.

Feed	In weight (g)	Fn weight (g)	PER	PI (mg)
F1	1.52± 0.11 ^a	12.45±0.03 ^a	1.23± 0.01 ^a	148.2 ^a
F2	1.53± 0.09 ^a	15.23±0.15 ^b	1.34± 0.02 ^b	181.3 ^b
F3	1.55± 0.07 ^a	18.12±0.17 ^c	1.4± 0.03 ^b	215.7 ^c

Means in a given column having the same letter superscript are not significantly different at (p <0.05) by ANOVA and Duncan multiple range test.

Table 7 – Survival percentage of *Catla catla* fingerling on every 4th week

Feed	Stocking Nos. (N=50 X 5 replicates)	4 th Week (%)	8 th Week (%)	12 th Week (%)
F1	250	88± 1.2 ^a	72± 1.8 ^a	60 ± 4.1 ^a



F2	250	91± 4.1 ^b	80± 3.2 ^{b,*}	70 ± 2.3 ^b .
F3	250	87± 3.4 ^a	84± 6.2 ^{c,*}	80 ±3.3 ^{c,**}

Same alphabet in superscript in a column represents no significant difference in survival. * = p< 0.01; ** = p< 0.05. The results are of five replicates of feeding trial.

Table 8 – Whole body composition of *Catla catla* fingerling

Feed	Dry Matter (%)	Crude Protein (%) [*]	Lipid (%) [*]	Ash (%) [*]	Organic Matter (%) [*]
F1	20.6± 0.45 ^a	48.4± 3.3 ^b	5.5± 0.2 ^a	12.1± 0.3 ^a	87.1± 1.3 ^a
F2	21.3± 0.27 ^a	45.1± 1.2 ^a	5.8± 0.4 ^b	11.4± 0.4 ^a	86.3± 1.5 ^a
F3	21.1± 0.33 ^a	46.2± 1.4 ^a	5.6± 0.1 ^a	11.3± 0.2 ^a	88.4± 1.0 ^a

Different alphabet in superscript in a column differ significantly (p< 0.05). The results are of five replicates of feeding trial. * = Dry matter basis

DISCUSSION

In the present study, the experimental feeds were formulations with different protein are based on previous reports (Kikuchi, 1999 and Cho et al., 2006). In the study, the experimental feeds F1, F2 and F3 with mustard oil cake, soybean and starch levels were formulated and the differences observed in the performance of the different feeds. Dietary proteins play a dominant role in fish growth (Cowey et al. 1972; Satia, 1974 and Cho et al., 1976). On the basis of average specific growth rate and % live weight gain, an improvement in growth response was noticed with increase in soyprotein (Das and Ray, 1991).

Results of this study substantiate the fact that various feed ingredients have direct growth promoting effects on Catla which is accordance with the report of Chaudhary and Qazi (2007). The overall growth pattern of fingerlings also remained highest for sunflower meal. The findings of Shabbir et al. (2003) are in agreement with the present study. They reported higher growth of *Cirrhinus mrigala* on sunflower meal, followed by maize gluten and wheat bran. Ali and Salim (2004) noted that *Labeo rohita* gained body weight on sunflower meal, which is less than the weight gained by Catla in the present study. The difference in weight gain may be due to variations in experimental fish and feed used. Ali and Salim (2004) used balanced feed, whereas in the present study four ingredients were used in the experiment. The present study showed that different protein types of even plant origin significantly affected the growth and feed utilization of Indian major Carp, Catla (*Catla catla*). As far as the value of FCR is concerned, the better (lower) feed conversion ratio was observed for sunflower meal, followed by cottonseed meal and bone meal. Ali and Salim (2004) noted higher FCR value for rice polish (5.27), followed by fish meal (3.026) and sunflower meal (3.021). The FCR values on rice polish meal were not comparable with present study. However, in the present study, FCR value on all the three rice polished mixed feeds were comparatively lower than the value observed by Ali and Salim (2004). Similar findings were also observed by Shabbir et al. (2003). The FCR value on cotton seed meal (1.55) reported by Jabeen et al. (2004) was somewhat lesser value of FCR observed in the present study. The negative effects of weight gain, FCR, PER in response to dietary plant protein from Mustard oil cake suggesting that dietary plant protein type from this origin is is poorly suitable than soybean protein. Similar reports are recorded in Japanese Flounder (Ye et al., 2011) by using soybean meal more than 16% and, who found that 43% of fishmeal protein could be replaced by soybean meal (25%) in combination with blood meal (10%) or corn gluten meal (10%) in blue murels meat @5% (Kikuchi, 1999).

The data in present study on *Catla catla* indicated that response to soybean meal protein substitution by mustard oil cake protein was somewhat better. According to, experiment conducted (Rao and Kumar, 2006) to know the effect of plant protein incorporated formulated feeds on the growth and nutritive value of Rohu fingerlings, the test feeds containing 35% dietary protein level, showed better performance in growth and fertilization than the control feed having only plant protein and also the test feeds having higher protein levels. This infers that the plant protein (MOC) can be replaced by Soybean meal (SBM), which is more efficient for growth promotion. Soybean meal has superior nutritive values over other plant proteins (Eyo, 1991), because of its well-balanced amino acid compositions and their bioavailability as reported on the influence of the performance of animal (Gaylord and Gatlin, 1996). The results of their findings are similar to our findings. Further, the foregoing results agree and extend the findings (Chakrabarty et al., 1973) by showing that groundnut and wheat bran was better utilized by fingerling *Labeo rohita* and *Cirrhinus mrigala* than that of mustard oilcake and rice bran. Prawn shell waste protein is rich in essential amino acids (Forster, 1975).

In the present experiment conducted to know the effect of different feed, containing plant proteins and also may be having anti-nutritional factors, and may lead to cumulative effects on growth performance in longer days feeding trials. Based on the results of the present study, it was concluded that mustard oil cake, potato starch, rice polish and soybean meal can be included in combination in the feed formulation for catla fingerling.

CONCLUSION

Results indicate that soybean incorporated feed was much acceptable than alternative plant protein source for the catla (*Catla catla*) however, the potential for including mustard oil cake protein in the feeds of fish need more evaluation.



ACKNOWLEDGEMENTS

The financial assistance to one of the author (PS) is from Uttar Pradesh Council for Science and Technology, Lucknow, UP, India vide grant No. G/Adm. UPCST (Dr. P.P.Srivastava)/2009-1140, is gratefully acknowledged. Authors are thankful to the Director NBFGR for providing facilities to conduct the experiments.

REFERENCES

- Ali, T and M Salim (2004). Studies on the growth response and feed conversion ratio (FCR) of *Labeo rohita* fingerlings fed on rice polish, fish meal and sunflower meal. *Int. J. Agri. Biol.*, 6(5): 914-917.
- Balogu, DO, HF Philips, A Gannam, OA Porter and J Handcock (1993). Sunflower seed feed supplemented affects on channel catfish, *Arkansas Farm Res.*, 42(3): 10-11
- Chakrabarthy RD, Sen PR, Kowtal GV (1973). Observations on the relative usefulness of different feeds for carp spawn and fingerling. *Journal of Inland Fishery Society of India* 5: 182-188.
- Chaudhary and JavedIqbal Qazi, (2007). Influence of a probiotic *Pseudomonas pseudoalcaligenes* Fermented Feed on Growth Performance of Rohu(*Labeo rohita*) Fingerlings. *Punjab Univ. J.Zool.*, v0l. 22(1-2), pp. 41-56, 2007.
- Cho CY, Slinger SJ, Bayley HS (1976). Influence of level and type of dietary protein, and level of feeding on feed utilization by Rainbow trout. *J Nutr* 106: 1547-1556.
- Cho SH, Lee SM, Park BH, Lee SM (2006). Effect of feeding ratio on growth and body composition of juvenile olive flounder *Paralichthys olivaceus* fed extruded pellets during the summer season. *Aquaculture* 251: 78-84.
- Cowey CB, Pope JA, Adron JW, Blair A (1972). Studies on the nutrition of marine flatfish. The protein requirements of plaice (*Pleuronectes platessa*). *British Journal of Nutrition* 45: 233-241.
- Das I, Ray AK (1991). Growth response and feed conversion efficiency in *Cirrhinus mrigala* (Ham.) fingerlings at varying dietary protein levels. *Journal of Aquaculture in the Tropics* 6: 179-185.
- Das, KM, and S D Tripathi. (1991). Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. *Aquat. Living Resour.* 11:187-192.
- De-Silva, S S and R M Gunasekera (1991). A cultivation of the growth of Indian Chinese major carps in relation to the dietary protein content. *Aquaculture*, 92(2): 237-241.
- Devaraj, KV, G Y Keshavappa and JK Manissery (1976). Growth of grass carp, *Ctenopharyngodon idella* fed on two terrestrial fodder plants. *Aquaculture and Fisheries Management*, 17: 123- 128.
- Erfanullah and Jafri AK (1995). Protein-sparing effect of dietary carbohydrate in diets for fingerling *Labeo rohita* *Aquaculture*. 136(3) : 331-339.
- Erfanullah and Jafri AK (1998). Effect of dietary carbohydrate to lipid ratio on growth and body composition of walking catfish (*Clarias batrachus*), *Aquaculture* 161:159-168.
- Eyo AA (1991) The utilization of isonitrogenous feed mixtures containing blood meal, fish meal, groundnut cake and soybean meal by fingerling mudfish *Clarias anguillaris*. In: *The Annual Report of National Institute of Freshwater Fish Research, Nigeria*, pp. 96-103.
- Forster JRM (1975) Studies on the development of compounded diets for prawns. In: *Proceedings of the first International Conference on Aquaculture Nutrition* (Price KS, Jr Shaw WN, Danberg KS eds.), pp. 229-248. Delaware Sea Grant College Program & US/Japan Aquaculture.
- Gaylord TG, Gatlin DM III (1996) Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture* 139: 303- 314.
- Gull, Y., M. Salim, K. Shahzad and U. Noreen (2005). Study on the growth performance and feed conversion ratio of *Labeo rohita* fed on soybean meal, blood meal and corn gluten 60%. *Indus J. Biol. Sci.*, 2(4): 556-562.
- Hassan, MA, A K Jafri, A S, Alvi, S. Rana and U. Nazura (1996). Dietary energy and protein interaction and approach to optimizing energy, protein ratio in Indian major carps, *Cirrhinus mrigala* (Ham.) fingerlings. *J. Aquacult. Tropics*, 10(3): 183-191.
- Inayat, L and M Salim (2005). Feed conversion ratio of major carp, *Cirrhinus mrigala*, fingerlings fed on soybean meal, maize gluten and maize. *Pakistan Vet. J.*, 25(1): 13-17.
- Jabeen, S., M Salim and P Akhtar (2004). Study on feed conversion ratio of major carp *Cirrhinus mrigala* fingerlings fed on cotton seed meal, fish meal and barley. *Pakistan Vet. J.*, 24(1): 42-46.
- Jena, J K, PK Aravidakshan, C Suresh, H K Muduli, S. Ayyappan and S Chandra (1998). Comparative evaluation of growth and survival of Indian major carps and exotic carps in raising fingerlings. *J. Aquacult. Tropics*, 13(2): 143-149.
- Jhingran, V. G. (1991). *Fish and Fisheries of India*. 3rd Ed., Hindustan Publishing Co., Delhi, India.
- Kikuchi K (1999) Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 179: 3-11.
- Mokolensang, J F, S Yamasaki and Y Onoue (2003). Utilization of Shochu distillery by-products for culturing the common carp *Cyprinus carpio* L. *On Line J. Biol. Sci.*, 3(5): 502-507.



- Rao LM, Kumar S (2006) Effect of squillameal on the growth and nutritive value of *Labeo rohita* (Ham.). Asian Fisheries Science 19: 215-225.
- Rao, NGS (1987). Studies on the growth of fry and fingerlings of selected carps fed on formulated fish feeds. Ph.D. Thesis Bangalore University, Bangalore, p. 170
- Sabapathy, U and Teo, LH (1993). A quantitative study of some digestive enzymes in the rabbitfish, *Siganus canaliculatus* and the sea bass, *Lates calcarifer*. Journal of Fish Biology, 42: 595-602.
- Saeed, M, M Salim and U Noreen (2005). Study of the growth performance and feed conversion ratio (FCR) of *Labeo rohita* fed on soybean meal, blood meal and corn gluten 60%. Pakistan Vet. J., 25(3): 121-126.
- Saha, AK and AK Ray (1998). Evaluation of *Chuni*, a commercially available low-cost cattle fodder, in the diet for rohu, *Labeo rohita* (Hamilton), fingerlings. Aquacult. Res. 29:761-768.
- Satia BP (1974) Quantitative protein requirements of rainbow trout. Prog Fish Cult 36: 80-85.
- Sen PR, NGS Rao, SR Ghosh, M Rout (1998). Observations on the protein and carbohydrate requirements of carps Aquaculture, 13 pp. 245-255.
- Shabbir, S, M Salim and M Rashid (2003). Study on the feed conversion ratio (FCR) in major carp *Cirrhinus mrigala* fed on sunflower meal, wheat bran and maize gluten 30%. Pakistan Vet. J., 23(1): 1-3.
- Ye J, Liu X, Wang Z, Wang K (2011) Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquacult Int 19: 143-153.



EFFECT OF MAXIGRAIN SUPPLEMENT ON GROWTH PERFORMANCE, ECONOMIC INDICES AND HAEMATOLOGICAL PARAMETERS OF HEAT-STRESS BROILERS FED THREE DIETARY FIBRE SOURCES

S.G. ADEMOLA ^{1*}, M.D. SHITTU ³, M.O. AYANSOLA ¹, T.E. LAWAL ² and G.O. TONA ³

¹Dept. of Animal Nutrition and Biotechnology, LAUTECH, Ogbomoso, Nigeria

²Dept of Animal Sci. & Fisheries Mgt, Bowen Univ. Iwo, Nigeria

³Dept of Animal Production and Health, LAUTECH Ogbomoso, Nigeria

*Email: ademolasadiq@yahoo.com; Tell: 2348034363429, 2348072322561, 2348038120605

ABSTRACT: The study determined effects of Maxigrain supplementation to 3 dietary fibres on growth performance, economic indices, tibia ash of broilers raised under daily heat stress (42^ocentigrade) of 4 hours. A total of 162 day old broiler chicks of Arbor Acres strain were divided into 6 treatments with 3 replicates per treatment of 27 birds. The fibrous ingredients were wheat offal, rice bran and corn bran. These were included in broiler starter and finisher diets at 3% and 20% respectively. Feed and water were supplied ad libitum. Birds in groups T1 (wheat offal), T3 (rice bran), T5 (corn bran) were fed unsupplemented diets. Diets in T2 (wheat offal), T4 (rice bran) and T6 (corn bran) were supplemented with Maxigrain® at 100mg/kg. The results showed Maxigrain addition to corn bran- and rice bran-diets significantly ($P<0.05$) improved feed conversion of heat stress birds. Heat stress chickens fed rice bran Maxigrain diet had better final liveweight and improved compressive strength than those fed enzyme wheat offal diet (final liveweight of 1758.9 versus 1566.67 g per bird and compressive strength of 4.75 versus 3.04 Newton percm²). Heat stress broilers fed rice bran enzyme supplemented diet had the best feed conversion, strongest compressive strength and achieved the highest profit ($P<0.05$). Birds consumed less of Maxigrain diets. However, the enzyme failed to improve final liveweight of heat stress chickens fed wheat bran diet.

Key words: Heat stress, enzyme, fibres, tibia, compressive strength, broilers

INTRODUCTION

Feed ingredients of plant origin and their by-products contain a number of components that are resistant to monogastric digestive enzymes because of lack of and/ or insufficiently of endogenous enzyme secretions (Ravindran et al., 1999). These components lower the utilization of other dietary nutrients leading to performance reduction. The incorporation of feedstuffs containing antinutritive factors may adversely affect the performance of poultry. The nutritional strategy used to address the antinutritive feedstuffs involved the use of feed enzymes that offer immense potential to overcome the problems. Degradation of non-starch polysaccharide (NSP) through the use of enzymes is the underlying mechanism to improve bird performance by releasing trapped nutrients within the cell and lowering digesta viscosity to enhance nutrient digestion and subsequent absorption (Classen and Bedford 1991, Bedford and Schulze 1998). Maxigrain® has been identified to optimize the use of non-conventional feed ingredients by improving weight gain and feed conversion ratio in broilers, improve litter quality and egg production as well as shell quality. It also reduces levels of dicalcium phosphate incorporation in the feed substantially (Polchem Innovative Solution, 2013). Rearing of broiler in the tropical environment usually expose birds to heat stress.

Stress is used to describe the use of non-specific responses or defense mechanism of the body when confronted with abnormal or extreme demand (Sahin et al., 2009). High ambient temperature (that induces heat stress) is of great concern in all types of poultry operations. It compromises performance and productivity through reduced feed intake and decreasing nutrient utilization, body weight (BW), growth rate, egg production, egg quality, hatchability, carcass characteristics and other important traits governing the prosperity of the industry are adversely affected by severe heat stress (Geraert et al., 1996). Heat loss in poultry is limited due to feathering and the absence of sweat glands. This leads to physiological changes that are accompanied by a change in hormonal status and a reduction in feed intake to reduce metabolic heat production (Teeter et al., 1985). Broiler not only eats less at high temperature, it also gain less per unit of intake, especially at temperature above 30°C (Daghir, 2009). Considerable attention is given to nutritional manipulation to manage heat stress in poultry. These include dietary

ORIGINAL ARTICLE



fortification with vitamins, minerals, fat and amino acids. Heat stress affects drastically the enzyme-kinetics (Yang and Wang, 2006), and consequently the rate of metabolic pathways (Schlesinger et al., 1997). Increased mineral excretion is one of the important consequences of heat stress in chicks. Belay and Teeter (1996) reported lower retention rates for phosphorus, potassium, sodium, magnesium, sulphur, magnesium, copper and zinc in broilers raised at high cycling ambient temperatures. The following hypothetical questions were stated to address the effects of heat stress in relation to utilization of dietary fibres and the use of feed enzyme to solve the problem of fibre utilization during high environmental temperature. To what extent will heat stress have on the capacity of broilers to properly handle dietary fibres and what effect will these fibres have on tibia bone characteristics under daily high environmental temperature? Will enzyme supplementation to dietary fibres for broilers exposed to daily high environmental temperature achieve better performance and improve tibia bone than those fed without the enzyme? Hence, this study investigated effects of Maxigrain® supplementation to 3 dietary fibre sources on growth performance, economic production indices, tibia ash and other tibia characteristics of broilers raised under heat stress of 4 hours daily.

MATERIALS AND METHODS

Experimental condition

The experiment was carried out with birds exposed to an environmental temperature of 42° centigrade for a period of 4 hours daily (12.00noon-4.00pm). Two automatic adjustable thermostat electric fan heaters with 2000W 220 voltage output (Ningbo Aipai Electric Company Limited Zhejiang, China) were hanged on the walls of the poultry pens (at 100cm from the floor of the pens). These electric heaters were used to achieve uniform distribution of 42°C for the birds.

Experimental design

A 2 by 3 factorial arrangement under completely randomized design (CRD) was adopted for the study. The 2 factors were fibre sources (wheat offal, rice bran and corn bran) and Maxigrain® supplementation (at 0, 100mg/Kg) to examine interaction effects of the factors on the measured parameters of broilers.

Management of experimental birds

The management practices included thoroughly disinfection of the pens and allowed the pens to rest for 2 weeks. The equipments were cleaned. The foot-dip disinfectant was prepared at the entrance of the pen. A total of 162 day old broiler chicks of Arbor Acres strain were randomly distributed to 6 dietary groups. Each treatment contained 3 replicates of 27 birds. Other normal management practices were also observed during the study such as proper vaccination and medication. Starter diets were fed to the birds for first phase of growth (0-4 weeks) and finisher diets for the last phase of their growth (5th -7th week). The birds were fed experimental diets and given water *ad libitum* throughout the study period of 7weeks under high ambient temperature of 42°C for 4hours daily (12.00-4.00pm).

Formulation of experimental diets

There were 6 experimental diets containing 3 fibres which were wheat offal (WO), rice bran (RB) and corn bran (CB). These fibrous ingredients were included in the broiler starter and finisher diets at 3% and 20% respectively (Table I). Treatment groups of T1 (WO), T3 (RB), and T5 (CB) were diets without enzymes. The experimental diets of T2 (WO), T4 (RB) and T6 (CB) were supplemented with Maxigrain® at 100mg/kg.

Cost analysis

Economic efficiency of growth was estimated using profit as a proportion of feed cost.

Maxigrain® composition

Each gram of Maxigrain® contained 10,000 IU cellulase, 200 IU beta-glucanase, 10,000 IU xylanase and 2500 FTU phytase. Maxigrain® is manufactured by Polchem Hygiene Laboratories, India.

Chemical analysis and structural measurement of tibia

Six samples of tibia bones per treatment were used for each of ash, physical and structural measurements. The collected tibia bones were autoclaved using the procedure described by Hall *et al.* (2003). Ash content of the tibia were estimated by ashing the samples at 550 °C for 5 h. The tibia bones were subjected to direct axial loads using Essay Universal Machine. The loads (Newton) caused structural compression in the bones. The physical measurements of tibia bones were carried out with the aid of measuring tape: diameter, length and breadth of the bones. The area of the bones was a multiplication of length and breadth of the bones. The compressive strength of tibia was determined using load (Newton) divided by the area of the bone which was expressed as Newton/cm². The ash and structural measurements of tibia bones were carried out at the Laboratory of Department of Animal Production and Health, as well as Photogrametry/Geodetic/Structural Engineering Laboratory of Civil Engineering Department respectively, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.



Statistical analysis

All data collected were analyzed using factorial analysis of variance under completely randomized design (SAS, 1999). Significant means were separated using Duncan option of the same software. A probability of 5 percent considered significant.

RESULTS

Feed intake and feed conversion of heat stress broiler starters were significantly ($P < 0.05$) influenced by the main effects of dietary fibre sources and levels of Maxigrain® supplementation (Table II). Maxigrain® supplementation decreased the feed intake and improved feed conversion of broiler starters exposed to daily 4 hours of heat stress. Interaction of the dietary fibre source and Maxigrain® significantly ($P < 0.05$) improved final live weight and weight gain of heat stress broiler starters fed rice bran at 3% in the diet. However, heat stress broiler starters fed wheat offal and corn bran Maxigrain® supplemented diets resulted in lower final liveweight and weight gain than their counterparts fed wheat offal and corn bran unsupplemented diets.

Dietary treatment significantly ($P < 0.05$) influenced final live weight and feed conversion of the 49th day old broiler finishers exposed to daily 4 hours of heat stress (Table III). Heat stressed broiler chickens fed rice bran Maxigrain® diet had the best final live weight and most efficient feed conversion. Broiler chickens fed corn bran Maxigrain® diet had slightly improved final live weight than those fed unsupplemented CB diet. Chickens fed wheat offal diet supplemented with enzyme had the least final live weight among the treatment groups. Furthermore, Maxigrain® addition to the rice bran- and corn bran- diets significantly ($P < 0.05$) improved the feed conversion of the birds at finisher phase. However, chickens fed unsupplemented- and enzyme- wheat offal diets had statistically similar feed conversion.

Heat stress broiler chickens fed wheat offal- and rice bran- diets without enzyme had significantly ($P < 0.05$) lower compressive strength than those that were fed Maxigrain® supplemented wheat offal and rice bran diets (Table IV). Furthermore, heat stress broiler chickens fed enzyme supplemented wheat offal diet had significantly ($P < 0.05$) lower diameter, area and load of tibia than their counterparts fed unsupplemented wheat offal diets. Diets did not significantly ($P > 0.05$) influence tibia ash content of heat stress broiler chickens.

Highest and lowest feed costs (#/kg weight gain) were observed in heat stressed broilers fed unsupplemented corn bran diet and those fed enzyme rice bran diet respectively (Table V). Maxigrain® significantly ($P < 0.05$) lowered feed cost (#/kg weight gain) for broilers fed these dietary fibres. Furthermore, Maxigrain® supplementation to rice bran diet achieved maximum profit and produced the best economic efficiency of growth for heat stress broiler chickens.

DISCUSSION

Addition of Maxigrain® may be responsible for lower feed intake experienced by heat stress broilers and the resultant improved final liveweight of broiler finishers. Maxigrain® could have improved the utilization of dietary fibres and other energy giving nutrients thereby lowering the amount of feed consumed by broilers fed enzyme supplemented diets than those fed unsupplemented diets. This agrees with the report of Sekoni et al. (2008) that Maxigrain® supplementation increases the retention of many vital nutrients and metabolizable energy. The effect of multiple-enzymes preparation (Maxigrain®) to improve the overall performance of broilers fed these dietary fibres was feasible even during heat stress in this study. Similar account was given by Yu and Chung, (2004) who reported that broilers responded to enzyme supplementation with greater magnitude in the hot season than in the cool season. This was also supported by Sekoni et al. (2008) that Maxigrain® supplementation significantly improved the dietary fibre retention under normal environmental condition. Esuga et al. (2008) recorded higher broiler weight gain in an experiment designed to evaluate enzyme (Maxigrain®) supplementation to graded levels of palm kernel meal. Addition of Maxigrain® to wheat offal- and corn bran- diets did not improve the final liveweight, weight gain, compressive strength and other tibia characteristics of heat stress broilers. This implied that the enzymatic profile in Maxigrain® preparation could not hydrolyse the non-starch polysaccharide (NSP) of wheat offal diet so as to make nutrients and minerals more available for the birds. In addition, exposure of broilers to heat stress may have hindered the capacity of these birds to secrete adequate endogenous secretion to utilize the nutrients in corn bran diet. Ademola et al. (2012) reported that Roxazyme G® corn bran diet produced the best feed conversion and maximum profit among those fed either Maxigrain® or Roxazyme G® supplemented diets containing each of corn bran, wheat offal and brewery dry grain. They also showed that both enzymes failed to improve the performance and profits of hens fed wheat offal diet under normal environmental condition.

Biochemical assessment of tibia bone ash did not show significant effect, however, physical and structural measurements revealed that tibia compressive strength and other tibia characteristics were significantly enhanced by Maxigrain® supplementation to rice bran diet. Addition of Maxigrain® to rice bran diet resulted in the maximum profit which is likely connected with the ease with which broilers were able to utilize rice bran. These results were in accordance with the findings of Kaczmarek et al. (2007) who reported a significant interaction between temperature of drying maize and enzyme supplementation on body weight gain in the growing and total periods. These results demonstrated that it was possible to positively affect the performance of birds fed dietary fibres exposed to heat stress by supplementing the diets with the appropriate fibre degrading enzyme. However, profit obtained from heat stress broiler chickens fed corn bran supplemented diet did not respond well to enzyme supplementation.



Table 1 - Ingredient composition of starter and finisher diets

Parameters	Starter diets						Finisher diets					
	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
Maize	59.54	59.54	59.25	59.25	58.97	58.97	54.25	54.25	50.38	50.38	48.77	48.77
Soybean meal	30.71	30.71	31.00	31.00	31.28	31.28	20.00	20.00	23.87	23.87	25.48	25.48
Wheat offal	3.00	3.00	-	-	-	-	20.00	20.00	-	-	-	-
Rice bran	-	-	3.00	3.00	-	-	-	-	20.00	20.00	-	-
Corn bran	-	-	-	-	3.00	3.00	-	-	-	-	20.00	20.00
Fixed ingredients*	6.75	6.75	6.75	6.75	6.75	6.75	5.75	5.75	5.75	5.75	5.75	5.75
Maxigrain®	-	+	-	+	-	+	-	+	-	+	-	+
Calculated Analysis												
Energy	3000.34	3000.34	3033.24	3033.24	3028.40	3028.40	2840.44	2840.44	3006.60	3006.60	2895.78	2895.78
Crude Protein (%)	21.06	21.06	21.00	21.00	21.00	21.00	17.81	17.81	18.00	18.00	19.00	19.00
Calcium (%)	1.50	1.50	1.50	1.50	1.50	1.50	1.28	1.28	1.26	1.26	1.28	1.28
Available P. (%)	0.63	0.63	0.71	0.71	0.63	0.63	0.60	0.60	0.64	0.64	0.58	0.58
Methionine (%)	0.44	0.44	0.34	0.34	0.35	0.35	0.30	0.30	0.32	0.32	0.31	0.31
Lysine (%)	1.15	1.15	1.15	1.15	1.16	1.16	0.97	0.97	1.00	1.00	0.98	0.98
Crude fibre (%)	3.43	3.43	3.59	3.59	3.66	3.66	4.11	4.11	4.98	4.98	5.05	5.05

*Vitamin mineral premix supplied the following vitamins and trace elements per kg diet: Vit. A 12500IU, Vit. D₃ 2500IU, Vit. E 40mg, Vit. K₃ 3mg, Vit. B₁ 3mg, Vit. B₂ 5.5mg, Niacin 5.5mg, Calcium Pantothenate 1.5mg, Vit B₆ 5mg, Vit B₁₂ 0.025mg, Folic Acid 1mg, Biotin 0.08mg, Mn 120mg, Choline Chloride 500mg, Fe 100mg, Zn 80mg, Cu 8.5mg, I 1.5mg, Co 0.3mg, Se 0.48mg and Antioxidant 120mg. Fixed ingredients (FIs) for starter diet contained 2.65% fish meal, 1.5% oyster shell, 0.25% salt, and FIs for finisher diet contained 2.1% fish meal, 1% oyster shell, 0.3% salt. Both diets also contained 0.25% vitamin premix, 0.1% meth. and 2% bone meal. - = unsupplemented group + = MAXI supplemented group



Table 2 - Growth performance of heat-stress broiler chicks fed 3 fibre sources and Maxigrain® (1-28 days, g/bird)

Parameters	T1	T2	T3	T4	T5	T6	SEM	Fibre	Maxi	Int.
	Wheat offal		Rice bran		Corn bran					
	-	+	-	+	-	+				
Final live wt	905.56	893.33	875.56	1000.01	955.57	888.33	31.6	NS	NS	S
Init live wt	58.81	58.81	58.81	58.81	58.81	58.81	0.07	NS	NS	NS
Weight Gain	846.75	834.52	816.74	941.20	896.76	829.52	31.6	NS	NS	NS
Feed Intake	1616.67 ^c	1254.70 ^d	1609.50 ^c	1276.50 ^d	2365.00 ^a	1921.23 ^b	53.60	S	S	NS
Feed conversion	1.92 ^c	1.50 ^d	1.97 ^b	1.36 ^d	2.66 ^a	2.31 ^c	2.74	S	S	NS

^{abcd} Means along the same row with different superscripts are significantly different. (P<0.05), FS+= fibre sources, + and - mean Maxigrain® supplemented and Unsupplemented diets respectively, S= significant, NS= not significant.

Table 3 - Growth Performance of heat-stress broiler finishers fed 3 fibre sources and Maxigrain® (29-49 day, g/bird)

Parameters	T1	T2	T3	T4	T5	T6	SEM	Fibre	Maxi	Int.
	Wheat offal		Rice bran		Corn bran					
	-	+	-	+	-	+				
FLW	1606.67 ^{bc}	1566.67 ^c	1553.33 ^c	1758.90 ^a	1638.90 ^b	1717.77 ^{ab}	42.26	NS	S	S
ILW	905.56	893.33	875.56	1000.01	955.57	888.33	31.60	NS	NS	S
WG	701.11	673.33	677.78	758.89	683.33	829.43	40.19	NS	NS	NS
FI	2733.60	2053.30	2745.60	2333.30	2977.80	2845.60	224.64	NS	NS	NS
FCR	3.86 ^{abc}	3.05 ^c	4.08 ^{ab}	3.07 ^c	4.39 ^a	3.44 ^{bc}	0.28	NS	S	NS

^{abcd} Means along the same row with different superscripts are significantly different. (P<0.05), + and - mean Maxigrain® supplemented and Unsupplemented diets respectively. FLW= final live weight, ILW= initial live weight, WG= weight, FI= feed intake, FCR= feed conversion ratio.

Table 4 - Tibia characteristics, compressive strength and ash content of heat-stress broiler chickens fed 3 fibre sources and Maxigrain®

Parameters	T1	T2	T3	T4	T5	T6	SEM	Fibre	Maxi	Int.
	Wheat offal		Rice bran		Corn bran					
	-	+	-	+	-	+				
Diameter (cm)	0.63 ^a	0.59 ^d	0.61 ^{cd}	0.62 ^{bc}	0.62 ^{ab}	0.61 ^{bc}	0.004	NS	S	S
Area (cm ²)	13.69 ^a	9.69 ^b	11.30 ^{ab}	8.16 ^b	9.38 ^b	9.30 ^b	0.99	NS	S	NS
Load (Newton, N)	36.00 ^a	29.20 ^b	31.50 ^{ab}	33.67 ^{ab}	35.00 ^a	36.00 ^a	1.47	NS	NS	S
Compressive strength (N/cm ²)	2.70 ^b	3.04 ^b	3.04 ^b	4.75 ^a	3.79 ^{ab}	3.63 ^{ab}	0.37	S	S	S
Tibia ash (%)	42.72	42.84	42.63	44.13	43.06	43.94	1.57	NS	NS	NS

^{abc} Means along the same row with different superscripts are significantly different. (P<0.05), S= significance, NS= not significant

Table 5 - Cost and profit of heat stress broiler chickens fed 3 fibre sources and Maxigrain®

Parameters	T1	T2	T3	T4	T5	T6	SEM	Fibre	Maxi	Int.
	Wheat offal		Rice bran		Corn bran					
	-	+	-	+	-	+				
Feed cost per Kg wt gain (#/kgWG)	405.55 ^c	354.77 ^{cd}	420.63 ^{bc}	307.18 ^d	548.34 ^a	458.03 ^b	24.66	S	S	NS
Profit (#kgWG)	294.45 ^{bc}	354.23 ^{ab}	279.37 ^{bc}	392.82 ^a	151.66 ^d	241.97 ^c	24.66	S	S	NS
EEG (%)	72.83 ^{bc}	100.66 ^{ab}	68.14 ^{bcd}	130.81 ^a	28.09 ^d	34.17 ^{cd}	12.94	S	S	NS

^{abcd} Means along the same row with different superscripts are significantly different (P<0.05), S= significance, NS= not significant. Total P.= Total period

CONCLUSION

Heat stress decreased growth performance and tibia compressive strength of broiler chickens fed these dietary fibres. Maxigrain® enhanced the growth performance, tibia compressive strength and profit obtained from heat stress broilers chickens fed rice bran diet. The study clearly showed that enzyme supplementation could be used to combat heat stress in broiler chickens based on specific fibre-enzyme interaction and reduced feed intake.

REFERENCES

Ademola SG, Egbewande OO, Lawal TE, Isah AT and Kuranga SM (2012). Effects of Roxazyme G® and Maxigrain® on performance, egg quality, cost-benefit and haematological parameters of laying hens



- fed wheat Offal, corn bran and brewery drygrain diets. *International Journal of Poultry Science* 11 (1): 33-38
- AOAC (2000). *Official Methods of Analysis*. 17th ed. AOAC International, Washington, DC.
- Bedford MR and Schultz H (1998). Exogenous enzymes for pigs and poultry. *Nutrition Research and Review* 11:91-114.
- Belay T and Teeter RG (1996). Effects of environmental temperature on broiler mineral balance partitioned into urinary and fecal loss. *British Poultry Science* 37:423-433.
- Classen H L and Bedford MR (1991). The use of enzymes to improve the nutritive value of poultry feeds. 95-116 In: *Recent Advances in Animal Nutrition*. Butterworth-Heinemann Ltd., Oxford, U.K.
- Daghir NJ (2009). Nutritional strategies to reduce heat stress in broilers and broiler breeders. *Lohmann Information* 44 (1) 6-15.
- Esuga PM, Sekoni AA, Omega JJ and Bawa GS (2008). Evaluation of enzyme (Maxigrain®) supplementation of graded levels of palm kernel meal (PKM) on the performance of broiler chickens. *Pakistan Journal of Nutrition* 7 (4): 607-613.
- Geraert PA, Padilha JCF and Guillaumin S (1996). Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. *British Journal of Nutrition* 75(2): 195-204.
- Hall LE, Shirley RB, Bakalli RI, Aggrey SE, Pesti GM and Edwards Jr., HM (2003). Power of two methods for the estimation of bone ash of broilers. *Poultry Science* 82:414- 418.
- Kaczmarek S, Cowieson AJ, Jozefiak D, Bochenek M and Rutkowski A (2007). The effect of drying temperature and exogenous enzymes supplementation on the nutritional value of maize for broiler chickens. 16th European Symposium on Poultry Nutrition, 555-558.
- Polchem Innovative Solution (2013). Cocktail enzymes- Maxigrain® retrieved on January 23, 2013 at <http://polchemgroup.com/index.php>.
- Ravindran V, Selle PH and Bryden WL (1999). Effects of phytase supplementation, individual and in combination with glycanase on the nutritive value of wheat and barley. *Poultry Science* 78(11) 1588-1595.
- Sahin K, Sahin N, Kucuk O, Hayirli A and Prasad AS (2009). Role of dietary zinc in heat -stressed poultry: A review. *Poultry Science* 2176-2183.
- SAS INSTITUTE (1999) *SAS/STAT User's Guide: version 8th* SAS Institute Inc., Gary, North Carolina.
- Schlesinger MJ, Ryan C, Chi MM, Carter JG, Pusateri ME and Lowry OH (1997). Metabolite changes associated with heat shocked avian fibroblast mitochondria. *Cell Stress Chaperones* 2:25-30.
- Sekoni AA, Omega JJ, Bawa GS and Esuga PM (2008). Evaluation of enzyme (Maxigrain®) treatment of graded levels of palm kernel meal on nutrient retention. *Pakistan Journal of Nutrition* 7(4) 614-619.
- Teeter RG, Smith MO, Owens FN, Arp SC, Sangiah S and Breazile JE (1985). Chronic heat stress and respiratory alkalosis: Occurrence and treatment in broiler chicks. *Poultry Science* 64:1060-1064.
- Yang PC and Wang CS (2006). Heat stress increases protein antigen transport across the intestinal epithelium via a mechanism of impairing proteolytic enzymatic activity. *Digestive Diseases and Sci.* 51:1003-1011.
- Yu B and Chung TK (2004). Effects of multiple-enzyme mixtures on growth performance of broiler fed corn-soybean meal diets. *Journal of Applied Poultry Research* 13:178-182.

THE ROLE OF THREE WILD ANIMALS IN THE DISTRIBUTION OF PREFERRED FORAGE PLANTS IN THE DINDER NATIONAL PARK (D.N.P) SUDAN

F. A. MOHAMMED¹, S. MAHGOUB², S. M. ABDEL HAMEED³

¹College of Veterinary Medicines and Animal Production Dept. of Fish and Wildlife Sciences, Sudan University of Science and Technology, Sudan.

²Ministry of Science and Technology, Forestry Research Centre, Seed Centre, Soba, Sudan

³Ministry of Science and Technology, Animal Resources Research Corporation, Wildlife Research Centre, Khartoum, North Sudan.

*Email: ¹ fat123ima@yahoo.com; ² Salwa39@hotmail.com; Tel & Fax: 00249 (85) 314798

ABSTRACT: This study was conducted in Dinder National Park (D.N.P.) of Sudan during the dry season (March, April and May). Waterbuck (*Kobus defassa*), warthog (*Phacochoerus aethiopicus*) and Tiang (*Damaliscus korrigum*) in D.N.P were chosen for this study. Seeds of *Acacia nubica*, *Acacia seyal* and *Piliostigma reticulatum* recovered from the fecal samples of waterbuck showed a highly increased rate of germination above the control. *Acacia polyacantha* and *Sesbania sesban* showed decreased rate of germination below the control. The germination rate of *Acacia siberiana* showed no positive effect (zero) versus the control. The germination rate of the seeds of *Ziziphus-spina-christi* remained more or less above the control (53% and 50%, respectively). The germination of seeds of *Ziziphus spina-christi* from fecal samples of warthog showed higher increased rate of germination. The results of this study confirmed that the three wild herbivores are grazers, but they shift their diets towards forbs, woody plants and fruits of leguminous trees during the dry season. Waterbuck, Tiang and Warthog they depended on the plant diversely around water collecting places in the park (Mayas) for their diets, but they selected other plant species from the surrounding. Also this study provides the information regarding food habits and feed requirements of these wild herbivores. Such information might help in the management of the habitat (Mayas) and the protection and sustainability of wild herbivores in D.N.P.

Key words: Dinder National Park (D.N.P.), Wild Animals, Forage Plants

INTRODUCTION

Fecal material accompanying vertebrate-dispersed seeds at deposition sites plays an important role in enhancing seed germination and seedling survival (Traveset et al., 2001). Passage of the seeds of fleshy-fruited plants through vertebrates' guts has varying results on germination behavior (Traveset, 1998). Vertebrate dispersal agents include birds, mammals, fish and reptiles. Among mammals, the major dispersers in tropical regions are bats and primates (Abrahamson, 1989).

Claudia et al. (1997) found mammals constitute an important spectrum of dispersal agents of *Prosopis flexuosa* (Fabaceae) through strategies that, although significantly affecting viability in some cases, make germination easy. Riyou et al. (2004) found that high-density herbivore species often play an important role in forest regeneration sika deer (*Cervus nippon yakushimae*) and that deer herbivory was important for preferred species.

The ingestion of seeds by vertebrates is an important process affecting the distribution, structure, and composition of plant communities (Fenner, 2000). Gut passage can break the dormancy of pondweed seeds (Santamaria et al., 2002). The possible interaction between gut passage and other dormancy-breaking processes (such as stratification or drought, Probert, 2000) can explain the great diversity of

Results obtained when analyzing the effects of gut passage on seed germination patterns (Traveset and Verdu, 2002). Interactions between animal behaviour (chewing, digestion), seed characteristics and germination success were positively related to seeds longevity, remarkably, to seed mass, seed shape and retention time of seeds In stomach of animals (Cosyns et al., 2005).

ORIGINAL ARTICLE



Two mechanisms that could determine how herbivores affect germination in dry-fruited plants. These are the mechanical and/or chemical scarification of the seed-coat, which may depend upon chewing behaviour and gut retention time (Fredrickson et al., 1997) or on the type of food ingested with seeds (Jones and Simao, 1987), and the effect of surrounding faecal material on germination and/or future seedling growth (Ocumpaugh et al., 1996).

Germination of most un-infested *Acacia* seeds without an external factor that scarify seed coat is very low or zero (Halevy, 1974). Passage through herbivores gut or artificial chemical or mechanical scarification of the seeds increases the seed germination level (Or et al., 2003). Lampery et al. (1974) found that germination of *Acacia* seeds ingested by impala, Thomson's gazelle (*Gazella thomsoni*), dikdik (*Madaqua kirkii*) and dorcas gazelle (*Gazella dorcas*) was found to range from 11 to 28 %. Miller (1995) found that the cumulative germination of ingested seeds to *A. tortilis* and *A. nilotica* retrieved from the stomach of Kudu exhibited greater germination (48 and 22% respectively) than control (7 and 3.5%, respectively), but lower germination than seeds retrieved from kudu's dung (60 % for *A. nilotica* and *A. tortilis*) (Miller, 1995).

Rhone and Ward (1999) showed that the germination levels of intact *A. raddiana* and *A. tortilis* seeds from dorcas gazelle (*Gazelle dorcas*) and Arabian Oryx (*Oryx leucoryx*) fecal matter were significantly higher than those of undigested seeds. Most experiments except that of Coe and Coe (1987) showed that ingestion by herbivores causes an increase in germination.

There was a positive effect of herbivore body mass on the germination of ingested seeds. This effect is presumably mediated through the allometric scaling of digestion time to herbivores body mass, which results in greater removal of the hard seed coat, thereby facilitating germination. This result indicates that species composition of the ungulates might have important consequences for the recruitment of *Acacia* trees (Robbins, 1993). The wild ruminant they had the unique ability to shift their dietary preference and select various vegetation types and plant species or plant portions in accordance with season and availability of plant biomass without affecting the nutritional quantity of their diet. Hence, habitat preferences vary seasonally depending upon the nutrient and water content of grasses and browse at the time of forage utilization (Evans, 1979).

Statistical analysis

Data's were analyzed by standard analysis of variance (SPSS) The treatment means were compared and Spearman's Rank Correlation using to test Least Significant Difference (LSD) procedures at 5% level and 1% (Gomez and Gomez, 1984).

MATERIALS AND METHODS

Sample Collection

Random sample of 10 and 27 Kg of Warthog and Waterbuck fecal pellets were collected from heaps of pellets deposited around dry from Mayas including Abdel Ghani, Samaaya, Musa and Ein El Shames during dry season 2004. Hundred Pellets were picked randomly from fecal samples and gently ground to release and check the type of the seeds. The numbers of the released seeds were counted and identified as percentage in the 100 pellets. A small number of pellets were collected for tiang from Samaaya maya.

Isolation, assessment and germination of undigested seeds

Seeds separated from pellets were germinated in the germination room of the National Seed Center of the Forestry Research Corporation (Soba) following ISTA Rules Standards (1993). The temperature and light were fixed (temperature 30°C, 12 h illumination from fluorescent lamps). Seeds were grown in plastic trays full with pure sand and arranged in a randomized block design which were watered daily. Four replicates of 25 seeds were used for each type of seed. Germination statuses were done and counted every 7 days. The counted seedlings were removed from germination trays. To find the number of seed per kilogram of pellet and seedling per kilogram the following formula was used (ISTA 1993):

$$\text{Number of seed /kg (N)} = \frac{\text{number of seed in kg pellets}}{\text{Number of Kg of pellets}}$$

$$\text{Number of seedling /kg} = \frac{\text{N X Germination rate}}{100}$$

RESULTS

Seeds of seven forage plants were separated from 27 kg pellets of Waterbuck. Six of these were legumes, (*Acacia siberiana*, *Acacia polyacantha*, *Acacia seyal*, *Acacia nubica*, *Piliostigma reticulatum* and *Sesbania sesban*), in addition to *Ziziphus spina-christi* which not legumes (Table 1). Only 50 seeds/ kg of woody plant (*Ziziphus spina-christi*) were separated from the 10 kg of fecal samples (Table 2) of Warthog.

The percentage of seed germination of three species (*Acacia nubica*, *Acacia seyal* and *Piliostigma reticulatum*) separated from pellets of waterbuck was about 733%, 128% and 77%, respectively, above control. The germination of seeds of *Acacia polyacantha* and *Sesbania sesban* separated from pellets of waterbuck about 12% and 40 % respectively. The germination of *Acacia siberiana* showed no positive effect (zero) and *Ziziphus spina-christi* (Table 3). Warthog showed a higher germination tendency, with rate (66% above control) for of seeds of *Ziziphus spina-christi* (Table 4). No seedlings germinated from whole pellets sowing except *Acacia seiberiana* and *Sesbania sesban* in the fecal samples of Waterbuck (Table 3, and 4).



The germination rate of seeds recovered from faeces of Waterbuck and Warthog showed different effect of seeds coat (Table 5). For the medium seed coat thickness, the germination rate was 55.00 % against 27.50% for the control. For the soft coated seed, germination rate was 45.50% against 75.00% for the control. For the hard coat, germination rate was 41.33 % against 25.66% for the control.

Table1 - Number of seeds / 27 kg of pellets of Waterbuck during the dry season from mayas in D. N. P.

Plant species	*Seed coat thickness	Number of seed /kg pellets	Seeds /kg pellets	**Number of seedling /kg pellets
<i>Sesbania sesban</i>	Soft	57	2.1	1.13
<i>Acacia polyacantha</i>	Soft	14	0.51	0.27
<i>A. seyal</i>	Medium	150	5.6	3
<i>Piliostigma reticulatum</i>	Medium	1357	50.3	26.65
<i>A. nubica</i>	Hard	40	1.48	0.74
<i>A. siberiana</i>	Hard	850	31.5	6.6
<i>Ziziphus-spina-christi</i>	Hard	28	1.04	0.55

*classes of seed coat thickness were recorded from Mahgoub, 2004 National tree seed centre Sudan.
 **% germinated seed x No of seed / kg pellets

Table 2 - Number of seeds / 10 kg of pellets of Warthog during the dry season from mayas in D. N.P.

Plant species	Control	Separated seeds from pellets %	Pellets %	Increased or decreased %
<i>Ziziphus-spina-christi</i>	50	83	0	+66

Table 3 - Germination percentage of different seed species of plants recovered from pellets of Waterbuck collected from D.N.P.during dry season

Plant species	Control	Separated seeds from pellets %	Pellets%	Increased or decreased %
<i>Acacia polyacantha</i>	60	53	0	- 12
<i>A. seyal</i>	25	57	0	+128
<i>A.siberiana</i>	21	21	21	Zero
<i>A. nubica</i>	6	50	0	+733
<i>Piliostigma reticulatum</i>	30	53	0	+77
<i>Sesbania sesban</i>	90	54	10%	- 40
<i>Ziziphus-spina-christi</i>	50	53	0	+06

*Increased or decreased % calculated: $\frac{\text{Separated seeds from pellets} - \text{Control}}{\text{Control}} \times 100$

Table 4 - Germination of percentage different seed species of plants recovered from pellets of Warthog collected during dry season against their contro

Plant species	Number of seed /10 kg pellets	Seeds /kg pellets	*Number of seedling /kg pellets
<i>Ziziphus-spina-christi</i>	50	5	41.5

*% germinated seed x No of seed / kg pellets

Table 5 - Germination rate (%) of the soft, medium and hard coated seeds recovered from fecal samples of waterbuck and warthog and control collected from D.N .P.

Seeds coat	Germination rate (%) (Mean \pm SD)	
	Fecal	Control
Soft	45.50 \pm 13.44	75.00 \pm 21.21
Medium	55.00 \pm 2.83	27.50 \pm 3.54
Hard	41.33 \pm 17.67	25.66 \pm 22.37
Total	46.42 \pm 13.16	40.28 \pm 28.41

DISCUSSION

The present result revealed that fruit of *Ziziphus spina-christi* occurred in the fecal samples of Warthog and undigested seeds showed high germination rate of this plant also Dorst and Dandelot (1993) found that Warthog consumed fruits. Warthog was also observed to show high activity in the surroundings of the Mayas including,



digging the ground during the dry season. This mechanism could help the biota species to fertilize the ground. Some wild ruminants, such as eland (*Taurotragus oryx*) and impala (*Aepyceros melampus*) are called "broad spectrum feeders" (Olsen and Hansen, 1977). The present finding seven seeds of plant species (six legumes and one not legume) were separated or undigested by Waterbuck. The fruit of *Ziziphus spina-christi* was found in the diet composition of Warthog. This could be used as basic information on distribution, vegetation structure with regard to frequency, availability and variability. Also Waterbuck has the major role in seed dispersal and germination.

This result agrees with Cosyns et al. (2005) and Or et al. (2003). Leguminous seeds ingested with pod often pass intact through the ruminant ingestion system (Lamprey, 1967 and Lamprey et al., 1974) because browse species played a major role improving feed for ruminants (domestic and wildlife) in arid and semi-arid regions, particularly during dry season when poor quality and quantity residues prevail (Hashim 1990; Lefroy et al., 1992). Passage through the digestive system of waterbuck and warthog reduced germination success of indigested seeds in some species (Gardener et al., 1993). These may be related to characteristics of chewing intensity, seed shape, type of physiology and morphology of digestive tract. *Acacia nubica*, *Acacia seyal* and *Piliostigma reticulatum* showed higher success of germination (50%, 57% and 53%, respectively) above control (6%, 25%, and 30%, respectively). Lamprey et al. (1974) found that germination of *Acacia* seeds ingested by implala, Tomosons gazelle (*Gazella thomsoni*), dikdik (*Madaqua kirkii*) and *Gazella dorcas* to range from 11 to 28%. On the other hand, Miller (1995) reported that germination of ingested seeds of *Acacia torilis* and *Acacia nilotica* from stomach of kudu exhibited greater germination (48% and 22%, respectively). Also similarly for *Acacia raddiana* and *Acacia tortilis* seeds from *Gazella dorcas* faeces showed higher germination than undigested seeds (29.8% and 13.5%, respectively) (Rohner and Ward, 1999). The germination of *Acacia polyacantha* and *Sesbania sesban* seeds was reduced or decreased (35.71% and 54.54%, respectively). This decrease might be because the seeds stayed longer in herbivores digestive system or damaged by chewing seeds or due to seed characteristics. Lamprey (1967) found that the germination of treated *Acacia polyacantha* to range from 0% to 27%. Miller (1995) found that growth varied depending upon the species of herbivores. Lamprey (1967) found that the germination of *Acacia* seeds is very low or zero. In this study seeds of *Acacia siberiana* and *Ziziphus spina-christi* ingested by waterbuck remained more or less the same (21% and 53.57%), as the control (21% and 50%, respectively). Lamprey (1967) found that germination of ingested *Acacia siberiana* ranged from one to two %. These findings could be explained in view of seed coat thickness, nature and kind of dormancy. In addition to other factors related to acids found in animal stomach, time the seeds remain soaked in the stomach juice and other food components ingested with seeds. Passage in the animal digestive tract improved germination. Bebawi and Mohamed (1985) divided seeds of *Acacia* spp to three arbitrary groups according to the level of dormancy explained in the degree of thickness of the seed coat. These groups are; the high dormancy *Acacias* with and the thick seed coats (*A. siberiana*, *A. nubica* and *A. nilotica* etc.), the medium dormancy *Acacia* with moderately thick coat (*A. seyal*, *A. albida*, *A. raddiana*). The third group is the soft coated *Acacia* seeds which include *A. polyacantha*, *A. senegal* and *A. mellifera*.

Results obtained are in agreement with this division because *Acacia siberiana* of the high dormancy group (thick coat) was not affected in addition to that seeds of which *Acacia* are heavy and had an oblong shape this will make the seed movement rather slow in the animal stomach shape (Mahgoub, 2002), while *A. polyacantha* of the soft coat group was harmed in-between the moderately thick coated *Acacias* (*A. seyal*, *Piliostigma reticulatum*). These are positively affected by animal stomach. *Acacia nubica* seeds, although have thick coat and high dormancy were positively affected.

This may be due to shape of seeds which is spherical, that allows for free rotation and movement in the animal stomach which helps in scarification of different parts of the seed coat (Miller and Coe, 1993).

Ziziphus spina-christi have mostly two seed / fruit one of them is dormant the other is not (Mahgoub, 2002). These might be due to the fact that the stomach juice has harmful effect on non dormant seed, but had a positive effect on the dormant ones by breaking their dormancy. *Acacia polyacantha* and *Sesbania sesban* are of the soft coat legume seeds which might explain the harmful effect of stomach juice on them that it penetrated the seed coat to the embryo. However, for *Sesbania sesban* this harmful effect on seed had a positive effect on the whole vegetation by limiting the frequency and richness of this species otherwise it may invade the whole area outlining other species. As for seeds grown within the pellets all seeds except *Acacia siberiana* did not germinate. This may be either due to the pellets chosen for germination contained no seeds or the atmosphere of the pellets surrounding the seeds was not suitable for germination. These could be confirmed by more future studies.

Ziziphus spina-christi seed recovered from pellets of Warthog showed higher germination rate (83%) against control (50%) and high than effect of stomach of waterbuck. This might indicate the important role of this animal in dispersal and germination process. The Warthog stomach juice might be the reason behind breaking the seed dormancy above 66% of control.

CONCLUSION

The processes of the digestive systems of the herbivores had positive effect on seed germination. This might have resulted from complex herbivores-specific interactions between animal behaviors (chewing) and seeds characteristics (size, seeds coat, shape). Therefore the Waterbuck, Tiang and Warthog have great role in the dispersal of seeds of forage plants in their habitats while they are wondering around and ultimately in the regeneration of natural vegetation of the park.



REFERENCE

- Abrahamson W (1989). Plant-Animal Interactions. New York: McGraw-Hill. 481 pp.
- Bebawi EF and Mahmed SM (1985). The pre-treatment of seeds of six Sudanese *Acacia* to improve their germination responses. *Seeds Science and Technology*.
- Claudia MC and Ricardo AO (1997). Dispersal and germination of *Prosopis flexuosa* (Fabaceae) seeds by desert mammals in Argentina. *Journal of Arid Environments* 35:707–714.
- Coe M and Coe C (1987). A field Guide to the Acacias of Kenya. Oxford University Press, Oxford.
- Cosyns E, Delporte A, Lens L and Hoffmann M (2005). Germination success of temperate grassland species after passage through ungulate and rabbit guts. *J. of Ecology*. 93:353-361.
- Dorst J and Dandelot P (1993). A Field guide to the larger mammals of Africa. London W6 8JB, Harper Collins, Pp. 173-174.
- Evans PGH (1979). Habitat preference of ungulates in closed savanna of central Africa. *Mammal.Rev.*9:19-32.
- Edwards GR and Crawley MJ (1999). Rodent seed predation and seedling recruitment in grassland. *Oecologia* 118: 288–296.
- Fredrickson, E. L.; Estell, R. E.; Havstad, K. M.; Ksiksi, T.; Van Tol, J. and Remmenga, M. D. (1997). Effects of ruminant digestion on germination of Lehmann love-grass seed. *Journal of Range Management*, 50: 20–26.
- Fenner M (2000). Seeds: the ecology of regeneration in plant communities. CABI Publishing, Wallingford, UK.
- Gardener CJ, Mclvor JG and Jansen A (1993). Survival of seeds of tropical grassland species subjected to bovine digestion. *Journal of Applied Ecology*, 30:75-85.
- Hashim IM (1990). Abundance, Seed pod nutritional characteristics, and germination of leguminous trees in South Kordofan. *J. Rang Management*, 43 (4).
- ISTA (1993). Seed Science and Technology.21- Supplementary-Zurich Switzerlanda.
- Jones RM and Simao Neto M (1987). Recovery of pasture seed ingested by ruminants. Seed of six tropical pasture species fed to cattle, sheep and goats. *Australian Journal of Experimental Agriculture*, 27:235–256.
- Lamprey HF (1967). Notes on the dispersal and germination of some tree seeds through the agency of mammals and birds. *East Africa Wildlife. J. 5: 179- 180*.
- Lefroy, E. C.; Dann, P. R.; Wildin, J. H.; Wesley-Smith, R. N. and McGowan, A. A. (1992). The trees and shrubs as sources of fodder in Australia. *Agro-forestry Systems*. 20: 117 - 139.
- Mahgoub S (2002). Studies on the physiological, environmental and biological factors affecting the germination of some forest tree species seeds. Ph.D. Thesis. University of Khartoum.
- Miller MF (1995). *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing by rodents. *African Journal of Ecology*. 33: 194–210.
- Miller MF and Coe M (1993). Is it advantageous for *Acacia* seeds to be eaten by ungulates. *Oikos*. 66:364-368.
- Ocupaugh WR, Archer S and Stuth JW (1996). Switch-grass recruitment from broadcast seed fed to cattle. *Journal of Range Management*, 49:368–371
- Olsen FW and Hansen, R. M. (1977). Food relations of wild free-roaming horses to livestock and big game, Red Desert, Wyoming *J. Range Manage*. 30:17:20.
- Or K and Ward D (2003). Three-way interactions between *Acacia*, Large mammalian herbivores and bruchid beetles-a review. *African Journal of Ecology*, 41 :(3) 57-265.
- Probert R J (2000). The role of temperature in the regulation of seed dormancy and germination. In: M. Fenner [ed.], *Seeds: the ecology of regeneration in plant communities*, 261–292. CABI Publishing, Wallingford, UK. 1.
- Riyu T and Yumoto T (2004). Effects of sika deer on tree seedlings in a warm temperate forest on Yakushima Island, Japan. *Ecological Research*. 19: 291–300.
- Rohner C and Ward D (1999). Large mammalian herbivores and the conservation of arid *Acacia* stands in the Middle East. *Conservation Biology*. 13:1162-1171.
- Santamaria L, Charalambidou I, Figuerola J and Green AJ (2002). Effect of passage through duck gut on germination of fennel pondweed seeds. *Archiv fur Hydrobiologie*. 156: 11-22.
- Traveset A (1998). Effect of seed passage through vertebrate frugivores guts on germination: a review. *Perspectives in Plant Ecology, Evolution and Systematic*. 1:151-190.
- Traveset A, Bermejo T and Willson M (2001). Effect of manure composition on seedling emergence and growth of two common shrub species of Southeast Alaska. *J. Plant Ecology*. 155:29-34.
- Traveset A and Verdu M (2002). A meta-analysis of the effect of gut treatment on seed germination. In D. J. Levey, W. R. Silva, and M. Galetti [eds.], *Seed dispersal and frugivory: ecology, evolution and conservation*, 339–350. CAB International, Wallingford, UK.



NUTRITIONAL COMPOSITION AND EFFECTIVE DEGRADABILITY OF FOUR FORAGE TREES GROWN FOR PROTEIN SUPPLEMENTATION

J. GUSHA ^{1*}, N. T. NGONGONI ² and T. E. HALIMANI ²

¹Department of Para-clinical Veterinary Studies, Faculty of Veterinary Studies University of Zimbabwe

²Department of Animal science, Faculty of Agriculture, University of Zimbabwe

*Email: jtgusha@gmail.com; jgusha@vet.uz.ac.zw

ABSTRACT: The chemical composition and ruminal degradability of dry matter (DM) and nitrogen (N) of four browse legumes (*Gliricidia sepium*, *C. calothyrsus*, *A. angustissima* and *Leucaena. pallida*) were evaluated. The in sacco degradability of protein and DM of the four browse legumes were determined using four mature Friesian- Holstein rumen-cannulated steers (440±20kg) live weight. The objective of this study was to evaluate the usefulness of the browse legumes using the nylon bag technique. Nylon bags with 3g samples of dried ground legumes (3mm screen) were incubated in the rumen. The incubation times were 0, 6, 12, 48, 72 and 120 hours in four cannulated Friesian-Holstein steers. The browse legumes were analysed for nutritive value in terms of dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), Ash, condensed tannin (CT), calcium (Ca²⁺) and Phosphorus (P). Dry matter degradability was significantly different ($P<0.05$) and *Gliricidia* was highest, followed by *L. pallida* then *A. angustissima* and *C. calothyrsus* in descending order. Crude protein degradability was significantly ($P<0.05$) lower than that of DM and was highest in *L. pallida*, *G. sepium*, *A. angustissima* and finally *C. calothyrsus* at the bottom. Effective degradability of DM in the rumen of the steers was highest with *G. sepium* (880g/kg DM at rumen outflow rate of 0.02/h) and least with *C. calothyrsus* (504g/kg DM) ($P<0.001$). Effective degradability of nitrogen was highest with *L. pallida* (645g/kg DM at outflow rate of 0.02/h) and least with *C. calothyrsus* (103g/kg DM) ($P<0.001$). The degradability profiles of these browse indicated that they can be used as alternative protein supplements.

Key words: Nylon Bag Technique, Degradability, Effective Degradability, Ruminal, Rumen Cannulated Steers, Rumen Outflow Rates

INTRODUCTION

Seasonal fluctuation in crude protein (CP) content of tropical grasses from 15% in December to 3% in May affects livestock production (Francis and Sibanda, 2001; Ngongoni et al., 2006). Nitrogen is the most limiting element in agricultural production, and its deficiency reduces the productivity of crops, pasture and animals. Multipurpose tree legumes are increasingly recognized for their capacity to enhance the productivity and sustainability of tropical agricultural systems, both in developed and developing countries of the world (Abia et al., 2006; Ngongoni et al., 2006; Mupangwa et al., 2003). Tree legumes can provide fuel wood, nutrient-rich mulch, erosion control and land stabilization as well as other products such as food and fencing materials for farmers (Abia et al., 2006; Mapiye et al., 2006). Tree legumes are usually long-lived, drought tolerant and require less maintenance, and therefore enhance the sustainability of farming systems. However, one of their major uses is as a source of protein-rich high quality forage in ruminants (Mupangwa et al., 2002; Abia et al., 2006). In developing countries, forage legumes contribute high protein herbage to supplement low protein crop residues and other mature poor quality feeds (Matizha, 2000; Mlay et al., 2005; Ajayi et al., 2007).

Nutritionally, the forage and browse legumes are superior forage in protein and mineral content (particularly calcium, magnesium, copper and cobalt.) even though their nutritive value falls slightly with age (Baloyi et al., 1997). Protein supplementation from these sources could enhance carbohydrate fermentation in ruminant feeding on low quality roughage (Ngongoni et al., 2007) and hence increase their digestibility (Cabrita et al., 2005) as well as voluntary feed intake (Mupangwa et al., 2002; Lazzarini et al., 2009). However, full knowledge of the nutritive value and the content of anti-nutritional factors of these browse legumes is an essential prerequisite to properly balance their use in ruminant animal nutrition. The legumes can provide the ruminants with rumen degradable nitrogen and undegradable digestible dietary nitrogen, which pass the rumen undegraded to the intestines

ORIGINAL ARTICLE



(Mupangwa et al., 2003). The objective of the research is to address the nutritive value and degree of degradation using the nylon bag technique Mehrez and Ørskov (1977).

MATERIALS AND METHODS

Feed samples

Four legume samples of *A. angustissima*, *C. calothyrsus*, *G. sepium* and *L. pallida* were grown at the University of Zimbabwe Farm. The altitude is approximately 1300m above sea level. The area is situated at 18°N and 30°E with an annual average rainfall of 800mm. The legumes samples for degradability studies were harvested from the farm, taking the leaves only. The legume samples were dried under shade in the Bio-assay Laboratory in the faculty of Agriculture, Department of Animal Science. During the air drying, the browse leaves were turned frequently to ensure even drying for six days. The sample were milled through a 3mm screen and packed in plastic bags awaiting chemical composition analysis and *in sacco* nutritive evaluation.

Rumen degradability studies

Animals and feeding: The animals were handled in accordance to animal welfare regulation of the country. Four mature Holstein-Friesian steer weighing 440±20kg surgically fitted with a rubber rumen cannula of 8.5 cm diameter were used to determine the degradability profile of browse legumes using the nylon bag technique Bhargava and Orskov (1987). The steers were not housed in individual pens but allowed to move freely in the Bio-assay Laboratory of the Department of Animal Science. The animals were dosed with a broad spectrum anthelmintic (Systamex, CAPS Veterinary PVT Ltd, Harare, Zimbabwe). The steers were feeding *ad libitum* their usual hay and were supplemented with 8kg concentrates of 3:1 maize to cotton cake with 11% crude protein so as to increase proteolytic microbes. The supplement was given to the animals three weeks before the beginning of the degradability studies. This was done for microbial stimulation as well as for adaptation. Fresh water was always available from automatic drinkers.

Rumen in sacco incubation of the samples

The rumen dry matter (DM) and protein degradability of the feed samples was determined using the nylon bag technique as described by Bhargava and Orskov (1987). Three grams of feed was weighed into each of the eight nylon bags of (9×13) cm and pore size of 49µm (Polymon, Switzerland) for each incubation time and were incubated in the rumen of four fistulated Holstein-Friesian steer. The nylon bags were put around a cork (8 bags per cork and 2 per each sample) and six corks were aligned along a flexible vinyl tube, 40cm long and 6cm outer diameter with a weight on the bottom part. Around the cork bags were tied with a nylon string and the bags were suspended in the rumen of each steer by attaching one end of the vinyl tube onto the cannula cap using nylon thread 25cm long. A cork with 8 bags representing the four samples was withdrawn from each animal at 6, 12, 24, 48, 72 and 120 hours Bhargava and Ørskov (1987). The nylon bags were then washed under running tap water to remove rumen debris from the bags and then machine washed for 30 minutes. The zero hour time of DM loss was determined by soaking weighed nylon bags containing the samples in cold water for one hour, followed by washing under running tap water and then machine wash for 30 minutes. The samples were then treated in a stomacher before analysis for 5 minutes per bag to reduce microbial contamination from the feed residues left adherent to the bags after rumen incubation. The residues in the nylon bags were dried in an oven at 60°C for 24 hours, weighed and thereafter analysed for nitrogen content.

Chemical analysis

Nitrogen content in the feed and in the residues was analysed by Kjeldahl method according to A.O.A.C (1990). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were analysed according to Goering and van Soest (1970). Total ash was obtained by igniting a dried sample in a muffle furnace at 600°C for 24 hours and calcium and phosphorous was determined by the EDTA method and spectrophotometer method, respectively. Condensed tannins were determined in the four legumes according to the method of Porter (1986).

Data and statistical analysis

Degradability of dry matter and protein of the different feed samples was calculated from the disappearance of DM or nitrogen (N) from the bags after washing (zero samples) and incubation into the rumen. The degradability characteristics of DM and crude protein (CP) of each feed was calculated according to the model of Ørskov and McDonald (1979) or McDonald (1981) equations respectively

$$P = a + b(1 - e^{-ct}) \quad (1) \quad \text{Ørskov and McDonald (1979)}$$

$$P = a + b(1 - e^{-c(t-t_l)}) \quad (2) \quad \text{McDonald (1981)}$$

Where; P = the proportion degraded at time t , a = water soluble proportion, b = not water soluble but potentially rumen degradable proportion, c = degradation rate of b proportion, t = incubation time, e = exponential constant, $a + b$ = potential degradable fraction and t_l = time lag. Effective degradability (ED) of DM and N was calculated using assumed outflow rates of 0.02, 0.05 and 0.08 per hour according to Ørskov and McDonald (1979).

$$ED = a + (bc/c+k) \quad (3)$$

Where; ED = effective degradability and a , b and c are constants as described in equation 1 and 2 above and k = is the passage rate. The potential degradability was calculated as $(a+b)$. The effective degradability of DM OM



and N was calculated using the assumed fractional outflow rate k of 0.02 and 0.05 per hour according to the equation of Ørskov and McDonald (1979). The experimental design used is a completely randomized design using the model:

$$Y_{ijk} = \mu + A_i + T_j + I_k + \bar{e}_{ijk} \quad (4)$$

Where; μ = overall mean, A_i = fixed effects of animal, T_j = forage species, I_k = incubation period and \bar{e}_{ijk} = error effects respectively. Analysis of variance (ANOVA) test was carried out on the disappearance of DM, OM, and CP value using Statistical Analysis System (SAS) (1998). The mean values so obtained were fitted to the equation of McDonald and Ørskov (1979) using SAS Institute (1998)

RESULTS

Chemical composition of browse legumes used

The chemical composition of the four browse legumes is presented in the Table 1. The crude protein (CP) contents were significantly different ($p < 0.05$) with *G. sepium* having the highest followed by *A. angustissima*, *C. calothyrsus*, and finally *L. pallida* in that order. The species were significantly different ($p < 0.05$) in Ash content, ADF and condensed tannins. The *C. calothyrsus* has the highest tannins of 102g/kg DM, which higher than other browse species. The highest Neutral detergent fibre (NDF) and acid detergent fibre (ADF) was found in *A. angustissima* and the lowest is in *G. sepium*. There was no significant ($p > 0.05$) difference in dry matter (DM) for all the treatment diets. Organic matter (OM) was significantly different ($p < 0.05$) between treatments and *G. sepium* has 817g/kg DM, *A. angustissima* has 884g/kg DM, *C. calothyrsus* has 870g/kg DM and *L. pallida* has 840g/kg DM.

Table 1 - Chemical composition (g/kg/DM) of browse legumes used in the degradability study

Diet	DM	OM	CP	ADF	NDF	CT	P	Ca ²⁺	Ash
Gs	924	817 ^b	305 ^a	248 ^c	214	26 ^c	2.8 ^a	30 ^a	107 ^a
Aa	931	884 ^a	265 ^b	339 ^a	257	64 ^b	1.3 ^b	17 ^b	46 ^c
Cc	912	870 ^a	227 ^c	293 ^b	222	102 ^a	1.3 ^b	24 ^{ab}	42 ^c
lp	909	840 ^b	218 ^c	295 ^b	231	55 ^b	2.9 ^a	21 ^{ab}	69 ^b
Significance	ns	***	***	***	ns	***	***	***	***
LSD	35	27	23	36	46	17	0.3	11	19
Grand mean	919	853	254	294	231	62	2.1	23	66

DM dry matter, Organic matter (OM), crude protein (CP), acid detergent fibre (ADF), Neutral detergent fibre (NDF), condensed tannin (CT), phosphorus (P), calcium Ca; *G. sepium* (Gs), *A. angustissima* (Aa), *C. calothyrsus* (Cc), and *L. pallida* (Lp); Means with same ^{abcd} superscripts in column are not significantly different ($P > 0.05$); LSD = least significant different; Significant level = 0.05***; ns= not significantly different

Degradability of dry matter

The mean rumen degradability constants **a**, **b**, **c** and effective degradability constants **P** for DM and CP are given in table 2 and 3 respectively. The rapidly degradable fraction (a) for DM was significantly different ($p < 0.001$). The following order was observed and the highest was in *G. sepium* (422g/kg), *L. pallida* (427g/kg), with *A. angustissima* (410g/kg) and lowest in *C. calothyrsus* (347g/kg). There was no significant difference ($P > 0.05$) in the slowly degradable dry matter (SDDM) content of *G. sepium* and *L. pallida* but the SDDM content of *A. angustissima* was greater $p < 0.05$ than that of *C. calothyrsus*. *G. sepium* had a significantly $p < 0.001$ higher rate of degradation than that of *L. pallida*, *A. angustissima* and *C. calothyrsus* respectively. However, *L. pallida* and *A. angustissima* did not show any difference in the rate of degradation ($p < 0.05$). The mean rates of degradation were 0.235, 0.037, 0.016 and 0.034/hr respectively, for *G. sepium*, *A. angustissima*, *C. calothyrsus* and *L. pallida*.

Table 2 - Dry matter and effective DM degradability (g/kg) of the four treatments

Browse legumes	a	b	c	a + b	ED k = 0.02	ED k = 0.05
Gs	422 ^a	497 ^a	0.235 ^a	919 ^a	880 ^a	832 ^a
Aa	410 ^a	430 ^b	0.037 ^b	840 ^b	689 ^c	593 ^c
Cc	347 ^b	354 ^c	0.016 ^c	701 ^c	504 ^d	433 ^d
Lp	427 ^a	476 ^a	0.034 ^b	903 ^{ab}	726 ^b	620 ^b
S.e	43.9	28.2	0.06	73.2	54.1	67.3

^{abcd} figures with the same superscripts in column are not significantly different ($P > 0.05$); a= quickly degradable fraction; b = slowly degradable fraction; c = rate constant; ED = effective degradability at outflow rate (k) of 0.02/h and 0.05/h; S.e = standard error; *G. sepium* (Gs), *A. angustissima* (Aa), *C. calothyrsus* (Cc), and *L. pallida* (Lp)

Crude protein (CP) degradability

The *in situ* CP degradation constants and effective CP degradability's of the four browse legumes are given in Table 3. The quickly degradable CP (QDCP) fractions of the legumes were significantly different. *G. sepium* (320g/kg) had higher ($p < 0.001$) QDCP content than *A. angustissima* (117g/kg), *C. calothyrsus* (111g/kg) and *L. pallida* (84g/kg). There was no significant difference ($p > 0.05$) between *A. angustissima* and *C. calothyrsus*. The slowly degradable crude protein (SDCP) contents were also dependent on the levels of tannins and ADF. *G. sepium*



and *L. pallida* had significantly different ($p > 0.05$) SDCP values, which were higher than that of *A. angustissima*. There was significant ($p < 0.05$) difference in SDCP between *A. angustissima* and *C. calothyrsus*.

Table 3 - CP and effective crude protein degradability (g/kg) of the four browse legumes

Browse legumes	a	b	c	a + b	ED k = 0.02	ED k = 0.05
Gs	320 ^a	564 ^b	0.016 ^a	884 ^a	571 ^b	456 ^b
Aa	117 ^b	57 ^c	1.308 ^c	174 ^b	173 ^c	172 ^c
Cc	111 ^b	-8 ^d	0.590 ^c	103 ^c	103 ^d	104 ^d
Lp	84 ^c	643 ^a	0.036 ^b	727 ^a	645 ^a	554 ^a
S.e	32	125	0.260	157	148	139

^{abcd} Figures with the same superscripts in column are not significant ($p > 0.05$). a = quickly degradable fraction; b = slowly degradable fraction; c = rate of constant; ED = effective degradability at outflow rate (k) of 0.02 and 0.05 per hour. S.e = standard error; *G. sepium* (Gs), *A. angustissima* (Aa), *C. calothyrsus* (Cc), and *L. pallida* (Lp)

DISCUSSION

Chemical composition

The crude protein content of all the treatment legumes studied in this project were in excess of the proposed as the minimum required for lactation (120g CP/kg DM) and growth (113g CP/kg) in ruminants (Francis and Sibanda, 2001). The values are slightly lower from those reported in other studies especially for *C. Calothyrsus* (Abia et al 2006). However, the results suggest that the studied legumes may be good for protein supplement in both lactating and growing ruminants. There are species differences in CP content of the studied legumes and thus could be attributed to different species differences Mupangwa, et al, (2003) and the differences in maturity of the harvested leaves (Baloyi et al., 2008). *G. sepium* leaves had a higher crude protein than others and the results were comparable to those obtained by Rekha et al. (2004) and Archimede et al. (2009).

CP and Dry matter degradability

A. angustissima and *C. calothyrsus* had lower QDDM and QDCP content than *G. sepium* and *L. pallida*. This could be attributed to higher levels of tannins which bind to microbes. Therefore from the study *G. sepium* and *L. pallida* can provide rumen microbes with adequate volatile fatty acid (VFA) and ammonia for host ruminant energy and microbial multiplication. The results for *G. Sepium* conform to those found by Gonzalez et al., (2003) and Keopasenht et al. (2004). The lower QDDM and QDCP content in *C. Calothyrsus* and *A. acacia* may be associated with increase in ADF content. These browse with low QDDM and QDCP may not be used in high producing ruminant as they may fall supply adequate degradable energy and nitrogen for volatile fatty acid production and ammonia respectively. The results show that *C. Calothyrsus* and *A. Angustissima* may not support ruminants at high fractional outflow rates. Higher levels of tannin in these two browses than other also may have also caused a decrease in degradability of forage because tannins cause precipitation of microbes and enzymes which are supposed to cause break down of the molecules as resulted by Makkar (2003).

The SDDM contents of the legumes were above the range of 355 to 457g/kg DM reported for others tropical herbaceous legumes (Mupangwa et al., 2003). *G. sepium* and *L. pallida* maintained a higher SDDM and SDCP content than *C. calothyrsus* and *A. angustissima*. The low degradability of forages with high ADF and tannins is due to reduced penetrative ability to rumen microbes through lignified plant cell walls and precipitations effects of tannins to microbes and enzymes (Makkar et al., 2003, Baloyi et al., 2008). The potential DM degradability values of these legumes are within the range of 485 to 870g/kg (Baloyi et al., 1997) but higher than the range of 532 to 740g/kg reported for other herbaceous tropical legumes (Mupangwa et al., 2003; Baloyi et al., 2008). The decline in potentially degradable DM content of *A. angustissima* and *C. calothyrsus* may be explained by increases in less degradable fractions accumulating in the forages such as lignin and tannins.

The rate of degradation (c) is important in determining effective degradation (a) well as rumen fill Macdonald et al. (2002). The rates of DM degradation were different between legumes. Baloyi et al. (2008) reported a decline in degradation rate of DM in cowpea and silver leaf with increasing in ADF and NDF. The high protein content and the fragility of legume cells walls and high proportion of readily digestible thin walled, non lignified mesophyll tissues of the tropical legumes (Cook et al., 2005) could have resulted in maintenance of high degradation rates of *G. sepium*. The effective DM degradability decreased with increase in ADF and NDF as well as with increase in tannins. *G. sepium* maintained the highest effective degradability with *L. pallida* and *A. angustissima* acacia in the intermediate and *C. calothyrsus* was the least. These differences could have been caused by the species variation in fibre content and levels of tannins.

The Metabolisable protein system based the evaluation of dietary protein on the concept of quickly (QDP) and slowly (SDP) degradable protein to meet the needs of rumen micro flora and undegradable dietary protein to meet host animal requirements. The QDN fraction in this research was between 84 to 320 g/kg and was lower than the range of 214 to 496 /kg DM reported by Mupangwa et al 2006, Baloyi et al 2008 for other tropical legumes. The differences may be due to species variation and ADF content as well as the level of condensed tannins. Stage of maturity and ratio of stem to leave can also cause this variation as browse legumes tend to have a high stem to leave ratio compared to herbaceous legumes (Mupangwa, 2003). The reduction in QDN content of legumes with



increasing maturity is due to a decline in the soluble cell contents while cell wall contents increase (Baloyi et al., 2008). The slowly degradable Nitrogen (SDP) content of the forage ranged from -8 to 643 g/kg DM. The variation in SDP of the legumes could be due to difference in stage of maturity. *C. calothyrsus* had the lowest value and a negative value of -8g/kg DM. This could be due to microbial attachment to the sample. High levels of tannins could have caused to microbes enzymes and amylase from saliva to stuck to the sample particles. The attached microbes cause an increase in N levels of the residue hence lowering the N degradation of the SDP (Makkar, 2003). The levels of condensed tannins and ADF could be the major causes of variation in degradation and bacteriostatic effects of some tannin.

Effective degradability is a measure of the proportion of legume DM that can be fermented in the rumen before it passes to the lower digestive tract or post ruminal sites. (MacDonald et al 2002). High tannin levels also cause a decrease in degradability of forage because tannins cause precipitation of microbes and enzymes, which hydrolyze molecules into smaller particle (Makkar, 2003). The higher the tannins may therefore have a beneficial effect (increasing bypass protein or decreasing ammonia loss) or detrimental effect (depressing palatability, decreasing number ammonia, decreasing post-nominal protein absorption) on protein availability (Abia et al., 2006). *G. sepium* and *L. pallida* can be feed to ruminants with high fermentable energy sources as they have high values of a and b fraction compared to *C. calothyrsus* and *A. angustissima*. In this project the negative values obtained for the quickly degradable fraction could be a result of microbial contamination. The (b) fraction for *C. calothyrsus* was negative, this could be due to its high tannins, which cause, microbes, microbial enzymes and amylase from saliva to stick to food particle to the extent that it was inseparable by stomach. This presented complications in calculating both partial and effective degradability since these are calculated from these constants.

CONCLUSIONS

There is clear evidence that browse legumes can be used with reasonable confidence, as substitutes for protein supplements in at least medium to low producing ruminants and can be suitable for supplementing beef during periods of nutritional bottle-necks. Legumes of *Gliricidia* species and *Leucaena* species are of higher quality and are more suitable supplements where quickly degradable protein supplements are required. Feeding trials of communal environments still need to be done to validate the asserted findings.

ACKNOWLEDGEMENTS

The author is grateful to the support offered by the department of Animal Science, University of Zimbabwe. The funds that supported this research were obtained from the University of Zimbabwe farm.

REFERENCES

- AOAC, (Association of Official Analytical Chemists) (1990). Official Methods of Analysis 15th edition 2200 Wilson Boulevard Arlington Virginia 22201. U.S.A.
- Abia JN, Ngongoni NT, Gandiya F, et al (2006). Effect of supplementing natural pasture hay with five *C. calothyrsus* provenances on the intake, digestibility, nitrogen balance and excretion of purine derivative by goat. Tropical and subtropical agro ecosystems 6 pp 65-71
- Ajayi FT, Babayemi OJ and Taiwo A A. (2007). Effects of *Stylosanthes guianensis* and *Aeschynomene histrix* on the yield, proximate composition and *in-situ* dry matter and crude protein degradation of *Panicum maximum* (Ntchisi). *Livestock Research for Rural Development Volume 19, Article #32* Retrieved December 13, 2012, from <http://www.lrrd.org/lrrd19/3/ajay19032.htm>
- Archimède, H, Gonzalez Garcia G, Despois P, Etienne T, Alexandre G, (2009). Substitution of corn and soybean with green banana fruits and *G. sepium* forage in sheep fed hay-based diets: effects on intake, digestion and growth. *J. Anim. Physiol. Anim. Nutr.*, 94 (1): 118-128
- Baloyi JJ, Ngongoni NT and Hamudikuwanda H, (2008) Chemical composition and ruminal degradability of cowpea and silver leaf desmodium forage legumes harvested at different stages of maturity. *Tropical and Subtropical agro ecosystems* 8
- Baloyi JJ, Ngongoni NT, Topps JH and Ndlovu P, (1997). Chemical Composition and Degradability of *Brachystegia Specifomis* (Musasa) Leaves and Stems Harvested Over 4 months from three Sites in Zimbabwe. In: *Animals Feed. Sci and Tech* 69:179-186.
- Bhargava, PR and Orskov ER (1987) (Eds) *Manual for one Use of the Nylon Bag Technique in the Evaluation of Feedstuff* The Rowen Research Institute, Aberdeen UK pp 1-20.
- Cabrita ARJ, Fonseca AJM, Dewhurst RJ, Sampaio CVP, Miranda MFS, Sousa GNS, Miranda IMF. and Gomes E. (2005). Nitrogen Supplementation of Corn Silages.1 Effects on Feed Intake and Milk Production of Dairy Cows. *J. Dairy Sci.* 86:4008-4019 © American Dairy Science Association, 2003.
- Cook BG; Pengelly, BC; Brown, SD. et al., (2005) *Tropical Forages* CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia
- Francis J and Sibanda S (2001) Participatory action research experiences in smallholder dairy farming in Zimbabwe. *Livestock Research for Rural Development* 13 (3): 1-6 <http://www.cipav.org.co/lrrd/lrrd13/3/fran133.htm>



- Goering HK and Van Soest PJ, (1970) Forage fibre analyses Agriculture handbook No 379 United state, Department of Agriculture 4-6
- Gonzalez D; Quintero-Moreno, A; Palomares, R; Rojas, N; Araujo, O ; Soto, G., (2003). Use of *G. sepium* in feed supplementation of crossbred heifers and its effect on growth and the onset of puberty Revista Cientifica, Facultad de Ciencias Veterinarias, Universidad del Zulia, 13 (1): 45-52
- Lazzarini I, Detmann E, Sampaio CB., Paulino MF, Filho SV., Souza MA, Oliveira FA. (2009). Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *R. Bras. Zootec.* [online]. 2009, vol.38, n.10 [cited 2013-03-04], pp. 2021-2030 . Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-
- Makkar HPS, (2003). Effects and fate of tannins in ruminants animal, adaptation to tannins and strategies to overcome detrimental effects of feeding tannins rich feeds. *Small Ruminants Research*, 49: 41-256.
- Mapiye C, Foti R, Mwale M, Chikumba N, Poshiwa X, Chivuraise C, Mupangwa JF (2006) Constraints to adoption of forage and browse legumes by smallholder dairy farmers in Zimbabwe. *Livestock Research for Rural Development* 18 (12) <http://www.cipav.org.co/lrrd/lrrd18/12/mapi18175.htm>
- Matizha, W. (2000) A Comparative Nutritive Evaluation of the Tropical Forage Legumes Grown Successfully by Farmers in Zimbabwe, Ph.D. thesis 2000 University of Zimbabwe, Harare.
- McDonald P, Edwards AR, Greenhalgh JFJ, Morgan CA. (2002.) *Animal Nutrition*, Sixth edition Longman UK
- Mehrez A. Z Ørskov E (1979) A study of the artificial fibre bags techniques for determining the degradability of feeds in the rumen. *Journal of Agricultural Science Cambridge* 88; 645-650
- Mupangwa JF, Ngongoni NT and Hamudikuwanda H (2003): Effects of stage of maturity and method of drying on *in situ* nitrogen degradability of fresh herbage of *Cassia rotundifolia*, *Lablab purpureus* and *Macroptilium atropurpureum*. *Livestock Research for Rural Development* 15 (5). Retrieved March 8, 113, from <http://www.lrrd.org/lrrd15/5/mupa155.htm>
- Mupangwa JF, Ngongoni NT, Daka DE and Hamudikuwanda H (2002). The effect of supplementing a basal diet of veld grass hay with increasing levels of velvet bean hay (*Mucuna pruriens*) on nutrient parameters in sheep. *Livestock Research for Rural Development* 14 (4) 2002.
- Ngongoni NT, Mapiye C, Mwale M and Mupeta B (2006) Factors affecting milk production in the smallholder dairy sector in Zimbabwe. *Livestock Research for Rural Development* 18 (05). <http://www.cipav.org.co/lrrd/lrrd18/5/ngon18072.htm>
- Ngongoni NT, Mapiye C., Mupeta B., Mwale M. and Chimonyo M. (2007). Potential of farm-produced crop residues as protein sources for small-medium yielding dairy cows. *African Journal of Agricultural Research* 2:309-317.
- Ngongoni N. T., Mapiye C., Mwale M. Mupeta B. and Chimonyo M. (2009). Sunflower Based Rations for Small-Medium Milk Producing Dairy Cows. *Pakistan Journal of Nutrition* 8 (4): 377-383, 2009.
- Ørskov E R and Macdonald I (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. *Journal of agriculture Science Cambridge* 92; 499-503dition, SAS institute Inc Cary NC, USA
- Rekha Kurup; Deshmukh, AB, Bhavana Wankhade; Udar, SA., (2004). Effect of feeding of gliricidia (*Gliricidia maculata*) tree leaves on performance of growing crossbred heifers. *Anim. Nutr. Feed Technol.*, 4 (2): 197-201
- SAS (1998) Statistical Analysis System Institute Inc. SAS user guide Statistical version 3rd edition



CORRELATIONS AMONG CONCENTRATIONS OF SOME METABOLIC HORMONES AND NUTRITIONALLY-RELATED METABOLITES IN BEEF COWS

J. K. DAMPTEY, F. Y. OBESE*, G. S. ABOAGYE and R. A. AYIZANGA

Department of Animal Science, University of Ghana, P.O. Box LG 226, Legon, Ghana

*Email: fyobese@yahoo.com

ABSTRACT: A study was conducted to investigate correlations among some metabolic hormones and nutritionally-related metabolites in plasma samples from sixteen multiparous Sanga cows raised extensively on natural pasture during early lactation. Blood was sampled from cows once every two weeks, from week 1 to 9 postpartum. The samples were processed for plasma and concentrations of the metabolic hormones and nutritionally-related metabolites were measured. Insulin-like growth factor-I (IGF-I) was positively correlated with insulin (0.377; $P < 0.001$) and glucose (0.249; $P < 0.05$), but negatively correlated with urea (-0.241; $P < 0.05$). Insulin was positively correlated with glucose (0.440; $P < 0.05$), total protein (0.262; $P < 0.05$), and albumin (0.242; $P < 0.05$), but negatively related with cholesterol (-0.279; $P < 0.05$). Leptin was correlated positively with total protein (0.338; $P < 0.001$) and albumin (0.351; $P < 0.001$). There was a positive correlation between glucose and total protein (0.410; $P < 0.001$) or albumin (0.425; $P < 0.001$), but the correlation with urea was negative (-0.291; $P < 0.01$). Total protein was positively correlated with albumin (0.682; $P < 0.001$), but negatively correlated with cholesterol (-0.561; $P < 0.01$). Furthermore, albumin was negatively correlated with creatinine (-0.294; $P < 0.01$), while cholesterol was positively correlated with urea (0.253; $P < 0.05$), and creatinine (0.294; $P < 0.01$). The positive relationships among the nutritionally-related metabolites and metabolic hormones suggests that the effect of alterations in energy balance and (or) protein balance on postpartum ovarian function could be mediated through changes in the secretory patterns of these metabolic hormones.

Key words: Body Condition Score, IGF-I, Ovarian Activity, Postpartum Period

INTRODUCTION

A number of metabolic hormones and nutritionally-related metabolites in the blood are involved in energy/protein homeostasis and reproductive function, especially the resumption of ovarian cyclicity in cattle during the postpartum period (Wettemann et al., 2003). The metabolic hormones include growth hormone (GH), thyroid hormones (thyroxine and triiodothyronine), insulin, insulin-like growth factor-I (IGF-I) and leptin. The nutritionally-related metabolites include glucose, cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), total protein, albumin, globulin, blood urea nitrogen and creatinine (Diskin et al., 2003). The changes in circulating concentrations of metabolic hormones and nutritionally-related metabolites are important signals of the metabolic state of the animal especially on the adequacy of nutrient supply in relation to nutrient utilization (Lindsay et al., 1993).

Positive correlations have been reported among IGF-I, insulin, and body condition score (BCS) in heifers and beef cows (Bishop et al., 1994; Vizcarra et al., 1998). IGF-I and insulin are physiologically linked and both increase with BCS. However, the regulation of each hormone individually may vary according to metabolic status, and the direction of change in body weight (Leon et al., 2004). Plasma concentrations of leptin were also positively correlated with insulin and glucose, but negatively correlated with concentrations of GH and NEFA in dairy cows (Block et al., 2001). Presently information is lacking on the relationships among metabolic hormones and nutritionally related metabolites in beef cows raised in pasture-based systems in the coastal savannah zone of Ghana.

The main objective of this study was to investigate the correlations among the concentrations of some metabolic hormones and nutritionally related metabolites in the plasma from Sanga cows. This should provide information on the nutritional and metabolic status to guide management decisions towards improved animal productivity.

MATERIALS AND METHODS

Location of study, animals and design



The study was conducted at the Animal Research Institute's Katamanso station located at Lat 05° 44' N and Long 00° 08' W in the Accra Plains of Ghana. The area has a bimodal rainfall pattern with the major wet season occurring from April to July and the minor season from September to November. The remaining months constitute the dry period. Annual rainfall and temperatures range between 600-1000 mm and 20°C to 34°C respectively. Sixteen multiparous Sanga were housed in an open kraal and grazed daily from 05.00 h to 10.00 h and 13.00 h to 16.00 h mainly on natural pastures comprising *Panicum maximum*, *Sporobolus pyramidalis*, *Vertiveria fulvibarbis*, *Griffonia simplicifolia*, *Baphia nitida* and *Milletia thonongii*. Water was provided twice daily; morning and evening. Cows were milked twice a day; morning and evening during the rainy season and once a day during the dry season. Mating was natural, with service bulls running freely with females all year round. Calves were weaned naturally (between 6 and 9 months of age). The animals were treated against ecto-parasites using a pour-on acaricide (Flumethrin 1% m/v) once a month during the dry season and fortnightly in the wet season. Treatment against endo-parasites was done using an anti-helminth (Albendazole 10%) once a month during the dry season and fortnightly in the wet season. Cows were treated against diseases as the need arose and vaccinated against Contagious Bovine Pleuropneumonia once a year. Cows and their calves were weighed monthly, using a scale. Body condition score of cows was determined weekly, using the 9-point score (1= very thin and 9 = obese; Nicholson and Butterworth, 1986).

Blood samples were collected from cows once every two weeks, from week 1 to 9 postpartum at 08:00 h by jugular venipuncture into a 10 mL heparinised vacutainer tube (BD Vacutainer Systems, Plymouth, UK). They were then placed on ice immediately after collection, and plasma was separated by centrifugation at 1800×g for 15 min at 4°C. Plasma was stored at -20°C, until assayed for IGF-I, insulin, leptin, glucose, cholesterol, total protein, albumin, globulin, urea and creatinine at week 1, 3, 5, 7, 9 postpartum. Plasma concentration of IGF-I was measured in duplicate by the chloramine-T radioimmunoassay (RIA) method described by Gluckman et al. (1983). Interference by binding proteins was minimized by acid-ethanol cryoprecipitation method validated for ruminants by Breier et al. (1991). Insulin concentration in plasma was measured by double-antibody RIA as described by Tindal et al. (1978) while, leptin concentration in plasma was measured in duplicate by double-antibody RIA method of Blache et al. (2000). The concentrations of the nutritionally-related metabolites were measured using the VITROS 950 Chemistry Systems Auto-analyser (Ortho Clinical Diagnostics, UK). Glucose was measured based on the method by Trinder (1969) and cholesterol was determined based on the enzymatic method by Allain et al. (1974). Total protein concentration was determined based on the biuret reaction (Dumas et al., 1981). Albumin concentration was determined based on the method by Dumas and Biggs (1972), while globulin concentration in the plasma was computed as a difference between total protein and albumin concentration (Mapekula et al., 2011). Urea determination was based on the method by Sampson et al. (1980), while creatinine determination was based on the method by Ambrose (1983).

Data analysis

The effect of week of observation on the concentrations of insulin, IGF-I, leptin, glucose, cholesterol, total protein, albumin, globulin, urea and creatinine during the postpartum period was analyzed using the analysis of variance procedure in SPSS v.16.0 (SPSS, 2007). The Pearson's partial correlation coefficients were calculated to describe linear relationships among the concentrations of metabolic hormones and nutritionally-related metabolites from week 1 to 9 postpartum using SPSS v.16.0 (SPSS, 2007).

RESULTS AND DISCUSSION

The concentration of insulin increased ($P<0.05$), while that of urea decreased as lactation progressed (Table 1). This might be due to an improvement in the energy balance status of cows during this period. During early lactation, the energy requirements for milk production and maintenance of a cow exceed the available energy from feed intake leading to a state of negative energy balance (Jorritsma et al., 2003). The energy balance status of cows, however improves as lactation progresses (Lucy, 2000).

Table 1 - Concentration of metabolic hormones and nutritionally-related metabolites during week 1 and 9 postpartum in Sanga cows

Metabolite	Postpartum period (weeks)					P-value
	1	3	5	7	9	
IGF-I (ng/mL)	18.0	15.5	16.9	21.4	21.6	0.442
Insulin (µU/mL)	3.51 ^b	4.10 ^{ab}	3.65 ^{ab}	4.18 ^{ab}	4.30 ^a	0.021
Leptin (ng/mL)	1.13	1.14	1.11	1.08	1.11	0.754
Glucose (mmol/L)	3.52	3.62	3.78	3.78	3.62	0.066
Cholesterol (mmol/L)	2.37	2.66	2.66	2.69	2.37	0.220
Total protein (g/L)	84.0	81.9	83.3	84.0	83.5	0.397
Albumin (g/L)	29.6	28.7	28.9	29.8	30.4	0.244
Globulin (g/L)	54.3	53.1	54.0	53.6	53.1	0.758
Urea (mmol/L)	7.49 ^a	6.78 ^{ab}	6.56 ^{ab}	6.07 ^b	5.99 ^b	0.020
Creatinine (µmol/L)	96.5	96.6	97.2	98.1	98.6	0.989

a,b: Means within a row with different superscripts differ significantly ($P<0.05$)



Partial correlation coefficients for plasma concentrations of IGF-I, insulin, leptin, glucose, total protein, albumin, total cholesterol, urea and creatinine during week 1 to 9 postpartum are shown in Table 2. IGF-I concentration was positively correlated with insulin ($r= 0.377$, $P<0.001$) and glucose ($r= 0.249$, $P<0.05$), but negatively correlated with urea ($r= -0.241$, $P<0.05$). This suggest that energy status or energy intake may regulate plasma concentrations of IGF-I, insulin and glucose in the same direction, whilst regulating IGF-I and urea concentrations in different directions. Circulating IGF-I, insulin and glucose concentrations have been found to influence ovarian function in cattle through the stimulation of gonadotrophin releasing hormone (GnRH) and luteinizing hormone (LH) secretion (Diskin et al., 2003; Wettemann et al., 2003). Ciccioli et al. (2003) observed a positive correlation between plasma IGF-I, insulin and glucose concentrations in Angus x Hereford primiparous cows. Also, plasma IGF-I was positively related with glucose and negatively correlated with urea in Holstein- Friesian cows (Zulu et al., 2002; Obese et al., 2009).

Insulin was positively correlated with glucose ($r= 0.440$, $P<0.05$) total protein ($r= 0.262$, $P<0.05$), and albumin ($r= 0.242$, $P<0.05$), but negatively correlated with total cholesterol ($r= -0.279$, $P< 0.05$). Insulin regulates the use of glucose, and therefore glucose uptake by cells depends on the hormone insulin (Wettemann et al., 2003). The release of GnRH from the hypothalamus is stimulated by the combined action of insulin and glucose (Arias et al., 1992). In early lactation, when cows are in negative energy balance, they may develop ketosis and experience depressed insulin and glucose levels, with elevated ketones, free fatty acids, and cholesterol in the blood (Schwalm and Schultz, 1976).

There is a positive relationship between nutrient intake and concentration of leptin in plasma of cattle, as increased plane of nutrition is associated with increased circulating leptin concentrations (Delavaud et al., 2002; Leon et al., 2004). The significant positive relationships between leptin and total protein ($r= 0.338$, $P<0.001$), and albumin ($r= 0.351$, $P<0.001$) in the present study suggest that nutrient intake will regulate leptin, total protein and albumin in the same direction.

Glucose is utilized by all animal cells to produce energy (Richards et al., 1995). In the present study, glucose concentration was significant and positively correlated with total protein ($r= 0.410$, $P<0.001$) and albumin ($r= 0.425$, $P<0.001$), but negatively correlated to urea ($r= -0.291$, $P<0.01$). The concentrations of glucose, total protein and albumin remained relatively constant during the postpartum period, while that of urea declined as lactation progressed (Table 1).

Table 2 - Pearson's partial correlation coefficient (r) among some metabolic hormones and nutritionally-related metabolites during weeks 1 to 9 postpartum in Sanga cows

Variable	IGF-I	Insulin	Leptin	Glucose	Total protein	Albumin	Total Cholesterol	Urea	Creatinine
IGF-I	-	0.377***	0.030	0.249*	0.064	0.219	- 0.046	- 0.241*	0.027
Insulin		-	0.066	0.440	0.062*	0.242*	- 0.279*	- 0.188	- 0.212
Leptin			-	0.207	0.338***	0.351***	- 0.141	- 0.080	0.036
Glucose				-	0.410***	0.425***	0.190	- 0.291**	0.054
Total Protein					-	0.682***	- 0.561***	- 0.202	- 0.014
Albumin						-	- 0.279	- 0.014	- 0.294**
Total Cholesterol							-	0.253*	0.294**
Urea								-	0.054
Creatinine									-

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Total protein was positively correlated with albumin ($r= 0.682$, $P<0.001$) and negatively correlated with total cholesterol ($r= -0.561$, $P<0.001$). Total protein reflects the total amount of proteins in blood plasma needed for the transport of lipids, hormones, vitamins, metals and enzymes. Albumin, a portion of total protein, is the main protein of blood plasma which serves as an early nutritional indicator of protein status (Agenas et al., 2006). Since albumin is the main protein of blood plasma, an increase in albumin concentration results in an increase in total protein. The poor nutritional status of cows in this study, evidenced by the low average BCS of 4.7 to 5.1 (Fig.1) on a 9-point score of Nicholson and Butterworth (1986), coupled with the possible increased demand for energy or glucose may lead to negative energy balance. Increased lipolysis due to low glucose concentrations to meet energy requirements will result in increased blood levels of low density lipoproteins (LDL). The elevated levels of plasma LDL concentrations in turn will increase the rate of synthesis of cholesterol, leading to high cholesterol concentrations (Meyer and Harvey 1998; Trail et al., 2004).

Albumin was negatively correlated to creatinine concentrations ($r= -0.294$, $p<0.01$). Creatinine concentration is influenced by factors including an animal's diet and muscle mass (Otto et al., 2000). Protein breakdown of cows in this study was due to their poor nutritional status and this increased the plasma creatinine levels relative to albumin concentration.

Total cholesterol was positively correlated with urea ($r= 0.253$, $P<0.05$), and creatinine ($r= 0.294$, $P<0.01$). Due to poor nutritional status and BCS of the experimental cows, relatively less fat was available to be metabolized to provide energy. Thus more protein was metabolized to meet the energy requirements and this elevated the urea and creatinine concentrations. Also, during periods of energy restriction, the shortfall in energy may be met by the catabolism of body protein, resulting in increased urea concentrations (Greenwood et al., 2002).



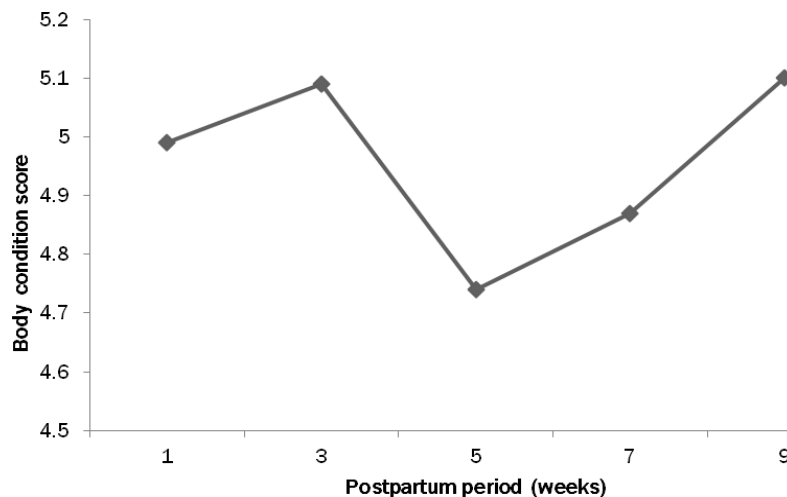


Figure 1 - Average body condition score of cows during week 1 to 9 postpartum in Sanga cows

CONCLUSION

There were positive or negative relationships among the concentrations of some of the metabolites measured. The positive relationships between changes in some energy and protein- related metabolites and metabolic hormones supports the assertion that the effect of alterations in energy balance and (or) protein balance on postpartum ovarian function could be mediated through changes in the secretory patterns of these metabolic hormones.

ACKNOWLEDGEMENTS

Mr. Mama Abdulai and Mr. Afranie of the Animal Research Institute (CSIR) are thanked for their assistance with blood sampling. Special thanks go to Mr. Tagoe of the Biochemistry Department and Mr. Ofori of the Central Laboratory of the Korle-Bu teaching Hospital for their assistance in determination of progesterone and blood metabolite concentrations.

REFERENCES

- Agenas S, Heath MF, Nixon RM, Wilkinson JM and Phillips CGC (2006). Indicators of under nutrition in cattle. *Animal Welfare*, 15: 149-160.
- Allain C C, Poon LS, Chan CSG, Richmond W and Fu PC (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, 20: 470 - 475.
- Ambrose RT, Ketchum DF and Smith JW (1983). Creatinine determined by "High Performance" Liquid Chromatography. *Clinical Chemistry*, 29: 256-259.
- Trail MA, Baker DC and Lassen ED (2004). *Veterinary Hematology and Clinical Chemistry*. Lippincott Williams & Wilkins. Maryland, USA. pp. 618.
- Arias P, Rodriguez M, Szwarcfarb B, Sinay IR and Moguilevsky JA (1992). Effect of insulin on LHRH release by perfused hypothalamic fragments. *Neuroendocrinology*, 56: 415 - 418.
- Bishop DK, Wettemann RP and Spicer LJ (1994). Body energy reserves influence the onset of luteal activity after early weaning of beef cows. *Journal of Animal Science*, 72: 2703-2708.
- Blache D, Tellam RL, Chagas LM, Blackberry MA, Vercoe PE and Martin GB (2000). Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. *Journal of Endocrinology*, 165: 625-637.
- Block SS, Butler WR, Ehrhardt RA, Bell AW, Van Amburgh ME and Boisclair YR (2001). Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. *Journal of Endocrinology*, 171: 339-348.
- Breier B H, Gallaher BW and Gluckman PD (1991). Radioimmunoassay for insulin-like growth factor-I: solutions to some potential problems and pitfalls. *Journal of Endocrinology*, 128: 347-357.
- Ciccioli N H, Wettemann RP, Spicer LJ, Lents CA, White LJ and Keisler DH (2003). Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. *Journal of Animal Science*, 81: 3107-3120.
- Delavaud C, Ferlay A, Falconnie Y, Bocquier F, Kann G and Chilliard Y (2002). Plasma leptin concentration in adult cattle: Effect of breed, adiposity, feeding level, and meal intake. *Journal of Animal Science*, 80: 1317-1328.
- Diskin MG, Mackey DR, Roche JF and Sreenan JM (2003). Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. *Animal Reproduction Science*, 78: 345-370.



- Doumas BT and Biggs HG (1972). Determination of serum albumin. *Standard Methods in Clinical Chemistry*, 7: 175-188.
- Doumas B, Bayse DD, Carter RJ, Peters Jr., T and Schafer R (1981). A candidate reference for determination of total protein in serum. 1. Development and validation. *Clinical Chemistry*, 27 (10): 1642-1650.
- Gluckman PD, Johnson-Barrett JJ, Butler JH, Edgar BW and Gunn TR (1983). Studies in insulin-like growth factor-I and-II by specific radioligand assays in umbilical cord blood. *Clinical Endocrinology*, 19: 405-413.
- Greenwood P, Hunt A, Slepatis, R, Finnerty K, Alston C, Beermann D and Bell AW, (2002). Effects of birth weight and postnatal nutrition on neonatal sheep. III. Regulation of energy metabolism. *Journal of Animal Science*, 80: 2850-2861.
- Jorritsma R, Wensing T, Kruip TAM, Vos PLAM and Noordhuize JPTM (2003). Metabolic changes in early lactation and impaired reproductive performance in dairy cows. *Veterinary Research*, 34:11-26.
- Leon HV, Hernandez-Ceron J, Keisler DH and Gutierrez CG (2004). Plasma concentrations of leptin, insulin-like growth factor-I, and insulin in relation to changes in body condition score in heifers. *Journal of Animal Science*, 82: 445-451.
- Lindsay DB, Hunter RA, Gazzola C, Spiers WG and Sillence MN (1993). Energy and growth. *Australian Journal of Agricultural Research*, 44: 875 - 889.
- Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. *Journal of Dairy Science*, 83: 1635-1647.
- Meyer DJ and Harvey JW (1998). *Veterinary Laboratory Medicine: Interpretations and Diagnosis*. 2nd Edn, Saunders, Philadelphia, USA. 392 pp.
- Mapekula M, Mapiye C and Chimonyo M (2011). Changes in metabolites concentration in Nguni and crossbred calves on natural pasture. *Asian- Australasian Journal of Animal Science*, 24(11): 1569-1576.
- Nicholson MJ and Butterworth MH (1986). A guide to condition scoring of Zebu cattle. International Livestock Research Institute (ILRI), Addis Ababa. 29 pp.
- Obese FY, Rabiee AR, Macmillan KL, Egan AR and Humphrys, S (2009). Effects of dry matter and metabolizable energy density on the concentrations of insulin-like growth factor-I and blood metabolites in pasture-fed dairy cows. *Ghanaian Journal of Animal Science*, 4: 68-77.
- Otto F, Baggasse P, Bogin E, Harun M and Vilela F (2000). Biochemical blood profile of Angoni cattle in Mozambique. *Israel Journal of Veterinary Medicine*, 55 (3): 1- 9.
- Richards MW, Spicer LH and Wettemann RP (1995). Influence of diet and ambient temperature on bovine serum insulin-like growth factor-I and thyroxine: relationships with non-esterified fatty acids, glucose, insulin, luteinizing hormone and progesterone. *Animal Reproduction Science*, 37:267-279.
- Sampson EJ, Baird MA, Burtis CA, Smith, EM, Witte DL and Bayse DD (1980). Coupled-enzyme equilibrium method for measuring urea in serum: optimization and evaluation of the AACC study group on urea candidate reference method. *Clinical Chemistry*, 26: 816-826.
- Schwalm, JW and Schultz LH (1976). Relationship of insulin concentration to blood metabolites in the dairy cow. *Journal of Dairy Science*, 59: 255-261.
- SPSS Inc. (2007). *SPSS for Windows version 16.0*. Chicago, Illinois, U.S.A.
- Tindal JS, Knaggs GS, Hart IC and Blake LA (1978). Release of growth hormone in lactating and non-lactating goats in relation to behaviour, stages of sleep, electroencephalographs, environmental stimuli and levels of prolactin, insulin, glucose and free fatty acids in the circulation. *Journal of Endocrinology*, 76: 333-346.
- Trinder P (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Annals of Clinical Biochemistry*, 6:24.
- Vizcarra JA, Wettemann RP, Spitzer JC and Morrison DG (1998). Body condition at parturition and postpartum weight gain influence luteal activity and concentrations of glucose, insulin, and nonesterified fatty acids in plasma of primiparous beef cows. *Journal of Animal Science*, 76: 927-936.
- Wettemann R P, Lents CA, Ciccioli NH, White FJ and Rubio I (2003). Nutritional- and suckling-mediated anovulation in beef cows. *Journal of Animal Science*, 81(E. Suppl. 2): E48-E59.
- Zulu VC, Sawamukai CY, Nakada K, Kida K and Moriyoshi M (2002). Relationship among insulin-like growth factor-I, blood metabolites and post-partum ovarian function in dairy cows. *Journal of Veterinary Medical Science* 64: 879-885.



EFFECT OF SURGICAL REMOVAL OF THE RESIDUAL YOLK SAC ON THE DEVELOPMENT OF THE DIGESTIVE SYSTEM AND IMMUNE RESPONSE IN BROILER CHICKS DURING EARLY DAYS POST-HATCH

H. A. ALI. OSAMA ^{1*} and E. E. MALIK. HUWAIDA ²

*E-mail: aburaghd69@yahoo.com

ABSTRACT: This study was designed to investigate the effects of the residual yolk-sac on the development of the digestive system and immune response of broiler chicks. Two experiments were conducted in this study. In the first one, 60 day-old broiler chicks (Lohmann) were allocated into three experimental groups according to the status of the residual yolk sac; deutectomized (surgically-removed residual yolk sac), sham operated and intact chicks. Five chicks from each experimental groups were randomly selected at days 2, 4, 6 and 8, weighed, euthanized and the different parts of their digestive tract and liver were weighed. The body weight of deutectomized chicks at day 2, 4 and 6 post-hatch was significantly ($P<0.05$) lower compared to that of sham operated and intact chicks. At day 8, the body weights of all experimental groups did not significantly ($P<0.05$) differ from each other. The liver weight in deutectomized chicks was significantly ($P<0.05$) lower at days 2, 4 and 6 post-hatch compared to that of the other experimental groups. At day 8 the liver weights in the different experimental groups did not show any significant difference. The weights of the different parts of the digestive tract (crop, gizzard, proventriculus, intestine), somehow, in deutectomized chicks were significantly ($P<0.05$) lower at day 2, 4 and 6 compared to that of sham operated and intact ones. In the second experiment, 60 day-old broiler chicks (Lohmann) were allocated to the above mentioned experimental groups; 20 chicks per each. Thereafter, they were challenged with 10% sheep RBC suspension at day 2 and day 12 post-hatch. Ten chicks were randomly selected from each experimental group at day 12 and day 20 post-hatching, killed and their lymphoid organs (spleen, thymus and bursa of fabricius) were incised and sera were harvested from blood samples. The lymphoid organs were significantly ($P<0.05$) lower in deutectomized chicks compared to the two other experimental groups. The geometric mean titers (GMT) of antibodies against 10% sheep RBC suspension for primary and secondary immune responses in deutectomized chicks, were lower than that of sham operated and intact chicks. The results of this study revealed that the residual yolk sac is essential for the development of the digestive system and immune response in broiler chicks.

Key words: Broiler Chicks, Deutectomy, Digestive system, lymphoid organs, Immune response.

INTRODUCTION

The avian species depend on the egg constituents to meet their requirements for growth and energy during embryonic life. Nevertheless, the significance of the yolk constituents extends beyond the embryonic life to cover the early days post-hatching. Its effects (as residual yolk sac, RYS) on growth of broiler chicks have been investigated by many studies (Chamblee et al., 1992; Turro et al., 1994 and Ali et al, 2007). Deutectomy has been shown to delay the growth of broiler chicks by 2 days (Chamblee et al., 1992), however, the chick can compensate for this delay by the end of the first week post-hatch (Chamblee et al., 1992 and Ali et al., 2007). Moreover, surgical removal of the residual yolk sac significantly decreases the liver total lipids during the first six days post-hatch in broiler chicks, which an effect lasts by the 8th post-hatch (Ali et al., 2011).

Feeding chicks immediately after hatching enhances the efficiency of yolk sac utilization (Bhanja et al., 2009). Noteworthy, broiler and layer chicks, subjected to feed and water deprivation showed striking differences in their efficiency of yolk utilization; it is significantly higher during the first two days post-hatch in broiler chicks (Malik et al., 2011).

Other biological functions of RYS have been also demonstrated; Olah and Glick (1984) reported that the RYS transforms to lymphoid tissues, exhibiting myelopoietic activity after complete absorption of the yolk contents.

The current study was designed to reflect more light on the physiological roles of RYS on post-hatch development of the digestive system and immune response of broiler chicks.

ORIGINAL ARTICLE



MATERIALS AND METHODS

Experimental Birds, Housing and Feeding

One hundred twenty commercial unsexed Lohman, day-old broiler chicks were employed in this study (60 chicks per each experiment). They were kept in a brooder house, at the Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum, where water and feed were provided *ad libitum*. Artificial and natural light was provided 24 hours a day.

Experimental plan

The chicks were assigned into 3 groups according to the status of the residual yolk sac; deutectomized, sham operated and intact chicks as follows:

a) **Deutectomized chicks:** The RYS was removed surgically within 4 hours post-hatching. Deutectomy was carried out according to a surgical technique described by Turo et al. (1994) with some modifications done to make the operation easier and more comfortable to the chick.

b) **Sham operated chicks:** In this group of experimental chicks, 5 mm surgical incision was made in the abdomen within 4 hours post-hatching. The incision was made in the same level of the umbilicus and just to the right side of it, and then it was sutured using silk.

c) **Intact chicks:** No incision or surgical operation was performed in this group of animals and they were kept as control.

Experiment 1

Sixty day-old broiler chicks were equally assigned into the 3 mentioned experimental groups. Five chicks were randomly selected from each group at day 2, 4, 6, and 8, weighed and euthanized by cervical dislocation. The alimentary tract was removed and its different parts were excised, emptied and weighed.

Experiment 2

After been assigned into the 3 mentioned experimental groups, the chicks were challenged with 1 ml of 10% sheep red blood cell suspension (SRBC, I/V) at day 2 and day 12 post-hatch. At day 12 (primary immune response) and day 20 (secondary immune response), 10 chicks from each group were randomly selected, weighed and euthanized. Blood samples were collected and sera were harvested and frozen for subsequent measurement of antibody titers against SRBC. This was performed using haemagglutination test as described by Singh and Dhawedkar (1993). The lymphoid organs (spleen, thymus and bursa of fabricius) were excised and weighed.

RESULTS AND DISCUSSION

The early days post-hatch is very critical for the subsequent development of chick; during which the body undergoes many metabolic changes. As it has been previously reported (Nitsan et al., 1991; Nir et al., 1993), the development of the digestive system and enzymes show continuous changes during this period. The residual yolk sac is the only extra-embryonic membrane exists during this transitional period and it has been shown, in addition to exogenous feed, to be critical for these changes (Gonzales et al., 2008; Ali et al., 2011).

Deutectomized chicks exhibited a significant ($P < 0.05$) decrease in the body weight at days 4 and 6, however, this effect was compensated at the 8th day post-hatch (Table 1). Previous findings showed that deutectomy did not influence the dietary energy use, lipids, and the carcass composition, but delayed the growth by 2 days behind the control chicks until the end of the first week, after which the chick can compensate for this delay (Murakami et al., 1992; Ali et al., 2007).

The current findings showed that deutectomy significantly ($P < 0.05$) decreased the weight of the liver (Table 2). Since deutectomy does not significantly affect serum total lipids (Ali et al., 2007) and free fatty acids (Baranyiova and Standara, 1980), it seems that the deutectomized chicks depends on the liver lipids stored during embryonic life. Noteworthy, the liver total lipids content is significantly affected by the interaction between days post-hatch and surgical ablation of residual yolk sac (Ali et al., 2007), which a finding support this assumption. Hence, considerable amount of fats might be drawn from the liver to the circulation to meet the energy needs of the deutectomized chicks. This might lead to a decrease in the liver weight exhibited by deutectomized chicks.

Deutectomy resulted in initial decrease in the weight of the different parts of the digestive tract, but this was compensated for by the 8th day post-hatch, at which the development of the digestive system was parallel to that of intact chicks (Tables 3-6).

The broiler chicks are characterized by high growth rate during the early days post-hatch due to a marked increase in the weight of the gastrointestinal tract (Nitsan et al., 1991), which is much higher as compared to that of the rest of the body (Nir et al., 1993). Post-hatch starvation results in a decrease in carcass lipid content but did not modify the disappearance rate of yolk in the abdomen (Murakami et al., 1992; Malik et al., 2011). Immediately after hatching, most energy and proteins are used for intestinal growth. This preferential growth occurs regardless of feed presence (Noy and Sklan, 1999; Maiorka et al., 2000). When these nutrients are not supplied by feed, newly hatched chicks use for intestinal growth the energy and protein from yolk sac. 20% of the residual protein of yolk



sac consists of maternal immunoglobulin, and the residual lipids of yolk sac are basically triglycerides, phospholipids, and cholesterol (Dibner et al., 1998). These components are used as intact macromolecules without metabolism, and the phospholipids and cholesterol are used for cell membrane formation (Maiorka et al., 2006). Nevertheless, it has been reported that nutrient supply from yolk is less crucial for the development of the digestive tract than withholding feed (Uni et al., 1998). Noteworthy, removal of the yolk sac reduces the activity of amylase, trypsin, and chymotrypsin in the intestinal chyme (Nitsan et al., 1995). Intubation with yolk increases enzyme activity (amylase excepted) in the pancreas or intestinal chyme only in chicks that had their yolk sacs removed (Nitsan et al., 1995).

The geometric mean titer (GMT) of antibodies against 10% sheep red blood cell suspension for primary and secondary immune response in deutectomized chicks was less than that of sham operated and intact chicks (Table 7). Moreover, surgical removal of the residual yolk sac significantly ($P<0.05$) reduced the weight of the lymphoid organs, (spleen, thymus and bursa of Fabricius), and this extended even after the completion of the course of the residual yolk absorption. By the 20th day post-hatch, the lymphoid organs weight in the deutectomized chicks were significantly ($P<0.05$) lesser compared to that of the other groups. These results are partially compatible with the findings of Thaxton (1984), who suggested that the yolk sac may play a role in clearance of antigens. Nevertheless, there are no previous findings supporting the direct effect of the residual yolk sac on the subsequent development of the lymphoid organs post-hatching.

Table 1 - Effect of deutectomy on the body weight (gm) of broiler chicks during first week post-hatch (Mean SEM)

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	47.82 ± 3.33 ^a	46.02 ± 1.92 ^a	42.42 ± 4.00 ^b
4	51.00 ± 4.30 ^a	53.80 ± 4.12 ^a	47.12 ± 3.00 ^b
6	58.42 ± 5.91 ^a	60.89 ± 5.78 ^a	52.68 ± 3.46 ^b
8	68.18 ± 6.82 ^a	71.50 ± 5.34 ^a	69.33 ± 4.89 ^a

Table 2 - Effect of deutectomy on the liver weight (gm) of broiler chicks during first week post-hatch

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	2.59 ± 0.12 ^a	2.47 ± 0.21 ^a	2.21 ± 0.23 ^b
4	2.73 ± 0.22 ^a	2.72 ± 0.31 ^a	2.16 ± 0.15 ^b
6	3.13 ± 0.42 ^a	3.04 ± 0.36 ^a	2.60 ± 0.15 ^b
8	3.04 ± 0.19 ^a	2.99 ± 0.34 ^a	3.17 ± 0.14 ^a

Table 3 - Effect of deutectomy on the crop weight (gm) of broiler chicks during first week post-hatch

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	0.76 ± 0.04 ^a	0.79 ± 0.04 ^a	0.72 ± 0.07 ^b
4	0.80 ± 0.03 ^a	0.77 ± 0.05 ^{ab}	0.68 ± 0.04 ^b
6	0.80 ± 0.04 ^a	0.75 ± 0.08 ^a	0.76 ± 0.03 ^a
8	0.73 ± 0.04 ^a	0.80 ± 0.03 ^a	0.63 ± 0.06 ^c

Table 4 - Effect of deutectomy on the proventriculus weight (gm) of broiler chicks during first week post-hatch.

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	0.81 ± 0.06 ^a	0.70 ± 0.03 ^b	0.74 ± 0.05 ^b
4	0.81 ± 0.04 ^a	0.87 ± 0.09 ^a	0.72 ± 0.04 ^b
6	0.94 ± 0.09 ^a	0.86 ± 0.04 ^a	0.73 ± 0.07 ^b
8	0.88 ± 0.11 ^a	0.89 ± 0.05 ^a	0.90 ± 0.04 ^a

Table 5 - Effect of deutectomy on the gizzard weight (gm) of broiler chicks during first week post-hatch.

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	3.26 ± 0.19 ^a	3.06 ± 0.09 ^a	2.68 ± 0.20 ^b
4	3.70 ± 0.28 ^a	3.35 ± 0.17 ^a	2.87 ± 0.12 ^b
6	3.56 ± 0.26 ^a	3.41 ± 0.21 ^a	2.96 ± 0.22 ^a
8	3.54 ± 0.39 ^a	3.59 ± 0.26 ^a	3.93 ± 0.29 ^a



Table 6 - Effect of deutectomy on the weight of empty intestine (gm) of broiler chicks during first week post-hatch.

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	2.80 ± 0.30 ^a	2.95 ± 0.22 ^a	3.05 ± 0.30 ^a
4	4.78 ± 0.27 ^a	4.71 ± 0.50 ^a	3.48 ± 0.34 ^b
6	4.97 ± 0.49 ^a	4.98 ± 0.25 ^a	4.45 ± 0.50 ^b
8	6.44 ± 0.39 ^{ab}	6.19 ± 0.26 ^b	6.74 ± 0.68 ^a

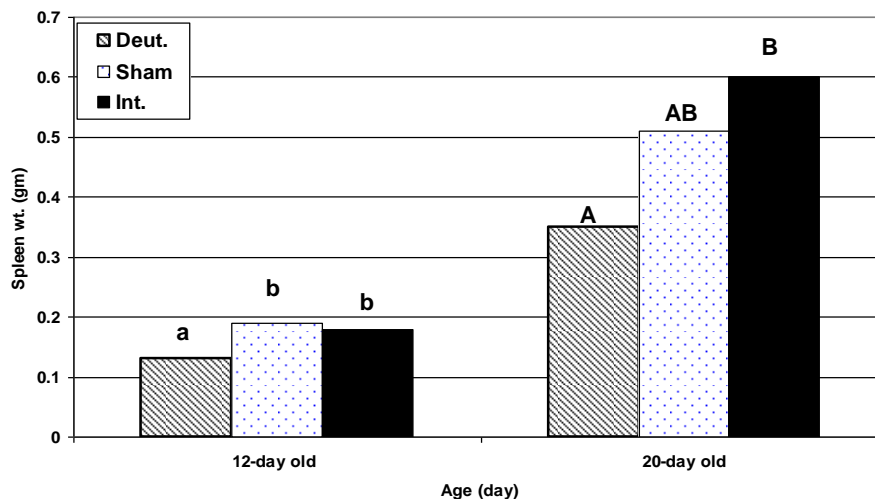


Figure 1 - Effect of deutectomy on Spleen weight (g) of 12- and 20-day old broiler chicks

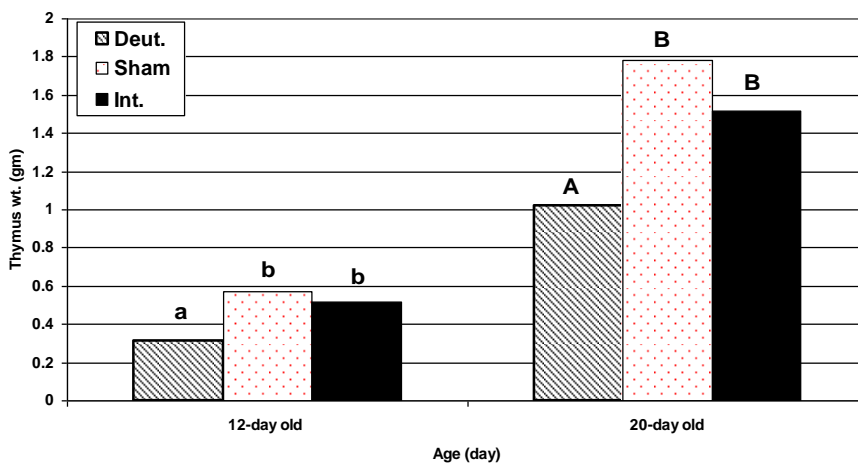


Figure 2 - Effect of deutectomy on thymus weight (g) of 12- and 20-day old broiler chicks

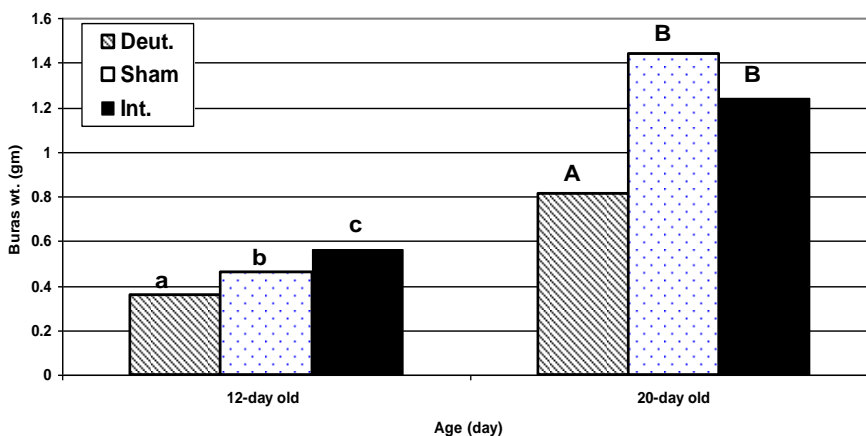


Figure 3 - Effect of deutectomy on Bursa of Fabricius weight (g) of 12- and 20-day old broiler chicks

Table 7 - Geometric mean titers of antibodies against 10% sheep RBCs suspension injected I/V in 2-day and 12-day old deutectomized, sham operated or intact chicks

Days post-hatch	Day 12 (primary immune response)	Day 20 (secondary immune response)
Status of residual yolk sac		
Deutectmized	2.5	5.3
Sham operated	4.3	8.0
Intact	4.6	8.6

CONCLUSION

This study showed that the residual yolk sac is very critical for the development of the digestive system and the immune response in broiler chicks during the early days pos-hatch.

REFERENCES

- AOAC (1984). Official methods of analysis of the association of official Agricultural chemists (OCLC) Number: 2438068
- Carew LB Machemer RH, Sharp RW and Foss DC (1972). Fat absorption by the very young chick. *Poult. Sci.* 51: 738-742.
- Chamblee TN, Brake JD, Schultz CD and Thaxton JP (1992). Yolk sac absorption and initiation of growth in broiler. *Poult. Sci.*, 71: 1811-1816.
- Frings SCS, Ted WF, Ralph TD and Cecelia A Q (1972). Improve determination of total serum lipids by the sulfo-phosphoanillin reaction: *Clinical Chemistry.* 18 (7): 673-674.
- Harvey JD, Parrish DB and Sanford PE (1955). Improvement in the technique of deutectomy of newly hatched chicks and the effect of the operation on their subsequent development. *Poult. Sci.* 34: 3-4.
- Huwaida EE Malik, OHA Ali, HM Elhadi and EA Elzubeir (2011). Residual yolk utilization in fast and slow-growing chicks, subjected to feed and water deprivation. *Asian J. of Biol. Sci.* 4: 90-95
- Kim E and Goldberg M (1969). Serum cholesterol assay using a stable liebrman-Burchard reagent. *Clinical Chemistry.* 15: 1171-1179.
- Maiorka A, Dahlke F, Morgulis M (2006). Broiler adaptation to post-hatching period. *Ciência Rural, Santa Maria,* 36(2): 701-708
- Murakami H, Akiba Y and Horiguichi M (1992). Growth utilization of nutrients in newly-hatched chick with or without removal of residual yolk. *Grwoth Dev. and aging.* 56: 75-84.
- National Research Council (NRC) (1994). Nutrient requirements of Poultry, 9th ed. National Academic Of science, Washington, DC.
- Nir I, Nitsan Z and Mahagna M. (1993). Comparative growth and development of the digestive organs and some enzymes in the broiler and egg type chicks after hatching. *Br. Poult. Sci.* 34:523-532.
- Nitsan Z, Duntington EA and Siegel PB (1991a). Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. *Poult. Sci.* 70:2040-2048.
- Nitsan Z, Avraham GB, Zorfe Z and Nir I (1991b). Growth and development of the digestive organs and some enzymes in the broiler chicks after hatching. *Br. Poult. Sci.* 32:515-523.
- Noy Y, Sklan D (1999). Energy utilization in newly hatched chicks. *Poultry Science,* 78: 1750-1756.
- OHA Ali, Elzubeir EA and Elhadi HM (2007). Effect of residual yolk sac on growth, liver total lipids and serum total lipids in broiler chicks. *Pak. J. Biol. Sci.* 10(24): 5059-5062.
- Olah I and Glick B (1984). Meekel's diverticulum. 1. Extramedullary myelopoiesis in the yolk sac of hatched chickens (*Gallus Domesticus*). *The Anatomical Record (Anat Rec.)*. 208(2): 243-52.
- Osama HA Ali, Huwaida EE Malik, and Hashim M Elhadi (2011) Changes in the concentrations of liver total lipids, serum total lipids and serum cholesterol during early days post hatch in broiler chicks. *Asian J. Poult. Sci.* 5 (1): 51-55
- Singh KC and Dhawedkar RG (1993). Immuno modulating effects of levamisole in chicks immunity compromised by infections bursal disease virus. *Trop. Anim. Hlth. Prod.* 25: 11-14.
- Thaxton JP (1984). Deutectomy and humoral immunity in neonatal broilers. *Poult. Sci.* 63: (suppl. 1): 41 (abstr.).
- Turro I, Dunnington EA, Nitsan Z, Picard M and siegal PB (1994). Effect of yolk sac removal at hatch on growth and feeding behaviour in lines of chickens differing in body weight. *Growth Dev. and aging.* 58: 105-112.
- Zelenka J (1973). Apparent digestibility of feed nutrients during the first days of chicken life. *Acta. Univ. Agri.* 21: 119-124.



PATHOGENIC MICROORGANISMS ISOLATED FROM PERIWINKLES IN CREEKS SOUTH-SOUTH OF NIGERIA

P. NWIYI¹, C. OKONKWO²

¹Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Micheal Okpara University of Agriculture Umudike Abia State, Nigeria

²Department of Veterinary Medicine, College of Veterinary Medicine, Micheal Okpara University of Agriculture Umudike Abia State, Nigeria

*Email: afodiokchukwu@yahoo.com

ABSTRACT: One hundred and twenty pieces of periwinkle were obtained each from Yenogoa and Oron Creek. The periwinkle were of two genera namely: *Pachymelania aurita* obtained from Oronk Creek located in Akwa-Ibom State, while the *Tympanotonus fuscatus* notably a brackish water habitat was obtained from Yenogoa in Bayelsa state both in south-south Nigeria. Evaluation of possible microbiological isolate was carried out according to Cowan and Steel's Manual for medical Bacterial identification. The Creek in Yenogoa presented high level of Coliform count $2.6 \times 10^5 \text{cfug}^{-1}$ while the Oron Creek had an unacceptable load of *Salmonella* count $6 \times 10^6 \text{cfug}^{-1}$. The total bacterial count was highest in Oron Creek $1.46 \times 10^8 \text{cfug}^{-1}$ from *Tympanotonus fuscatus*. The microorganisms isolated from both Creeks were *Esherichia coli*, *proteus sp*, *salmonella sp*, *pseudomonas sp* and *Enterobacter sp*. *Proteus sp* was the least isolated while *Salmonella sp* was the highest.

Key words: Pathogenic Microorganisms, Periwinkles, South-South, Nigeria.

INTRODUCTION

Periwinkles are marine mollusks found commonly in mangrove swamps, lagoons and estuaries and consist of two general *Tympanotonus* and *Pachymelania* (Buchaan, 1954). Periwinkles are mass consumer products (Ekanem and Otti, 1997). There are very cheap source of protein in Oron, Akwa-Ibom state and Yenogoa in Bayelsa state south-south Nigeria. Some are found mostly in shallow waters and sometimes in inter-tidal zones where they burrow into the mud in the beds of the river which serves as their habitat (Okon, 1987). The best method to process periwinkles before consumption differs among the populace. Some believe that the shell should be removed and the meat washed thoroughly before cooking, others think otherwise. However, studies on the microbiological quality of shell fishes have shown that they harbour many pathogenic microorganisms (Ukpong and Efuk, 1992). Most times, the accumulation and concentration of pathogenic microorganisms and other toxic materials are usually from untreated human waste and industrial effluents that find their way into the water bodies that are inhabited by these shellfishes (Montgomery and Needselman, 1992). Periwinkles have been implicated in outbreaks of food-borne disease in many parts of the world. These illnesses includes hepatitis, typhoid fever and other digestive disorders Metealf et al. (1973) and (Ekanem and Adegoke, 1995). Shellfishes concentrate in water bodies and industrial waste, hence the likeliness of high pathogen level and toxic contaminants which can present health hazard to consumers Longree (1990).

The need to inform the public on the health hazard associated with the consumption of periwinkle with its shell cooked which could result in ingestion of pathogenic microorganism's lead to this study. The aim of this study is to isolate and identify comparatively the possible microbial organisms present in *T. fuscatus* and *P. aurita* from the two sources and to evaluate their safety on consumption.

MATERIAL AND METHOD

Sample collection of periwinkle *T. fuscatus* and *P. aurita* was carried out in Oron and Yenogoa in the Creeks these areas are notable for producing periwinkle. One hundred and twenty samples of sixty each was collected from Oron and Yenogoa measuring 40.52 – 45.80 mm in length and weighing 3.80 - 5.56g in weight were obtained from earthen pond of Brackish water. They were kept in a plastic aerated container and transported to the Umudike veterinary microbiology laboratory for analysis. The periwinkle were divided into two groups, those obtained from Oron is designated as sample X why that of Yenogoa is sample Y.

The periwinkles were washed, scrubbed and rinsed and the meat carefully extracted from the shell using forceps as described by ALPHA (1970). The analyses were done in triplicate on 60g raw periwinkle samples which were blended with 460ml of sterile peptone water 0.1% as described in the Bacteriological Analytical Manual plates were prepared from 10 fold dilutions in nutrient agar (micro master) for total bacteria counts. MacConkey agar (micro-master) for total Coliform count and Salmonella/Shigella agar (Micro-master) for Salmonella – Shigella counts were made after incubation at 37°C for 24 hours. Colonies were characterized and identified using various morphological and biochemical tests such as mortality, catalase, urease, citrate, indole, oxidase MR - vP and sugar fermentation tests. The isolates were identified according to Cowan and steel's manual for Medical Bacteria Identification (Cowan, 1985).

RESULTS AND DISCUSSION

Table 1 shows the total microbial counts of periwinkle samples from two different swamps of Oron and Yenogoa. The total microbial load for Oron was 1.14×10^8 and 1.24×10^8 respectively from *T. fuscatus* represented as Y and Z while Yenogoa swamp total microbial load was 1.36×10^8 and 1.44×10^8 represented by W and X for *P. aurita*. The salmonella count recorded from Oron swamp 6×10^6 represented the highest microbial load compared to that from Yenogoa swamp which was 2.0×10^6 . This means that the swamp from Oron was more contaminated than that from Yenogoa and hence will support the growth of more microorganisms, this is in agreement with Nester et al. (1983). The Coliform count was higher in Yanogoa creek as represented 2.6×10^5 than 1.8×10^5 from Oron creek. There are high levels of pollution such as defecation, bathing, sewage discharge of effluent and washing in the fresh water environment than in brackish water where *P. aurita* is often found (Jay, 1986). The incidence of microorganism and other shell fish depends on the quality of water from which animals are obtained. This was supported by (Ekanem and Adegoke, 1995). The organisms isolated from these periwinkles: salmonella, proteus, enterobacter, Escherichia and Pseudomonas are very important bacteria that are of public health implications. Organisms like *Escherichia coli* have been associated with infantile diarrhea while enterobacter causes septicemia. Salmonella causes paratyphoid in humans and is in agreement (Nester, 1995). Through periwinkles are cheap source of protein, it has the tendency of harboring pathogenic microorganisms especially those relevant to human health due to the poor sanitary condition of the water bodies where these animals are cultivated and this is in agreement of the findings Adebayo-tayo et al. (2006).

Bacterial contamination in the periwinkle species from the two swamps exceeded the acceptable limits for shellfish. The International Commission on Microbiological Specifications for Foods ICMSF (1982) and the US Food and Drug Administration FDA (1991) have suggested a maximum microbial count of not greater than $1 \times 10^5 \text{ cfug}^{-1}$ and Coliform level of not greater than $1 \times 10^2 \text{ cfug}^{-1}$ of shellfishes for consumer safety. The result obtain here is in agreement with that reported by Ekanem et al. (1994).

Table 1 - Total microbial counts of periwinkle samples in Oron and Yenogoa

Location	Sample	Salmonella count (cfug ⁻¹)	Colliform count (cfug ⁻¹)	Total Bacterial count (cfug ⁻¹)
Oron	Y	6×10^6	1.6×10^5	1.46×10^8
	Z	1.2×10^6	1.8×10^5	1.24×10^8
Yenogoa	W	1.5×10^6	2.4×10^5	1.36×10^8
	X	2.0×10^6	2.6×10^5	1.44×10^8

Key Y and Z; *Tympanotonous fuscatus* , W and X; *Pachymelania aurita*

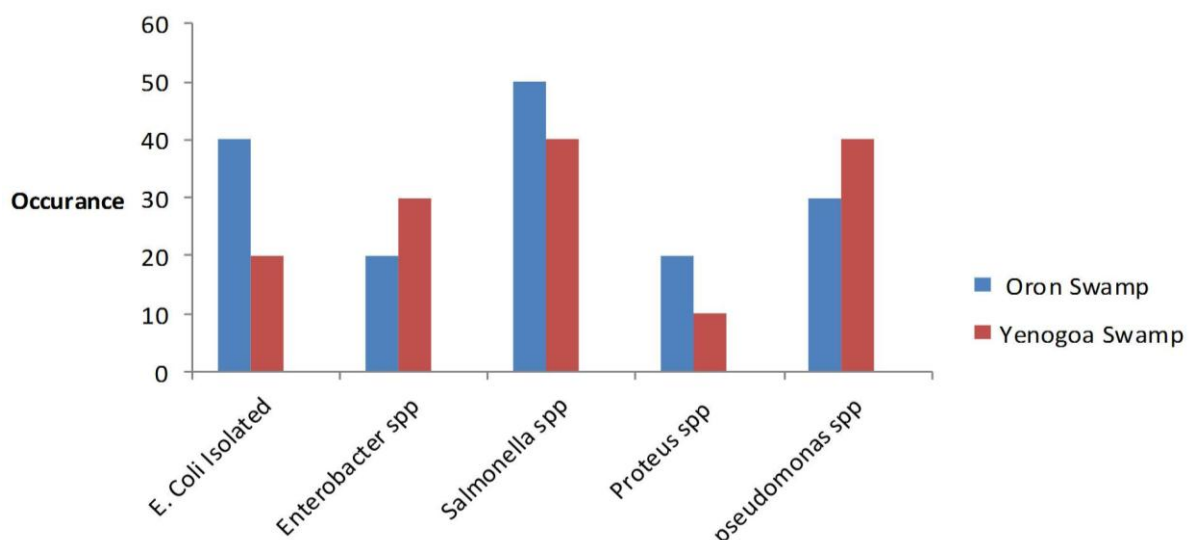


Figure 1 – Bactrial isolated from Periwinkle



CONCLUSION

The study evaluated the Oron and Yenogoa swamps (Creeks) for periwinkles produced there and the proximate bacteriological analysis represented. The samples from both Creeks presented an unacceptable volume of microbial organisms which is negative to human populace that consumes them. Hence, proper attention should be paid towards proper processing, handling and storage in order to minimize the risk to human infection on consumption.

REFERENCES

- Adebayo-Tayo BC, Onilude AA, Ogunyobi AA and Adejoye DO (2006). Bacteriological and proximate Analysis of periwinkles from two Different Creeks in Nigeria. *World Applied Science Journal*.
- ALPHA (1970). Procedure for the bacteriological examination of sea water and shell fishes. American public Health Association Washington DC.
- Buchaan JB (1954). Marine Mollusks of Gold Coast of West Africa Journal. West Africa Science Association 7:30-45.
- Cowan SY (1985) Cowan and Steels Manuel for the identification of medicated Bacteria (3rd Edition).
- Ekanem EO and Adegoke GO (1995). Bacteriological study of West Africa Clam (*Equaria radiata* Lamarch) and iterin overlying waters. *Food Microbiology* 12:381-385.
- Ekanem EO and Otti BN (1997). Total plate count and Coliform levels in Nigerian periwinkles from fresh and brackish water. *Food control*, 8: 87-89.
- Ekanem EO, Adegoke GO and Ossai GL (1994). Bacteriological and organoleptic characteristics of dehydrated West African Dam meat. *Nigeria Food Journal* 12:66-73.
- FDA (1991). Sanitation of shellfish Growing Areas and seafood safety (F.E Ahmed). National Academic Press Washington DC.
- International Commission of Microbiological Specification for Food. ICMSF (1982). Micro-organisms in Foods. Their significance and methods of enumeration. 2nd Edition. University of Toronto Press. Toronto 2: 19-30.
- Jay JM (1986). Modern Food Microbiological (3rd Edition) Van Nostrand Reinhold New York.
- Longree K (1990). Quality Food Sanitation (3rd Edition). John Wiley and Sons Jnr. New York. 1:121-127.
- Meteaff TG, Slanetz LW and Bartley CH (1973). Enteric pathogens in estuary water and shellfish: In microbial safety of fishery products. Chichester, C.O and Graham H.D. (Editions).
- Motgomery M and Needelman M (1997). Board of Reagent of the University of Wisconsin System (BRUNS). The welfare of Toxic Contaminants in Fresh water fish.
- Nester EL, Roberts CE, Lidstrum ME and Pearsale NN (1983). Microbiology, 3rd Edition, Saunders college Publication. Holt, Japan 1: 888-889.
- Nester EW (1995). Microbiology, A human perspective W.M.C Brown Publishers. Oxford, England.
- Okon BI (1987). Utilization of periwinkles flesh by Broilers feed palm kernel-based rations. Ph.D thesis University of Ibadan, Nigeria.
- Ukponge E and Utuk O (1992). Microbiological Quality of *Equaria radiata* in Cross River, Book of Abstracts Nigeria. Institute of Food Science Technology 1:15-16.



GROWTH PERFORMANCE AND HAEMATOLOGICAL PARAMETERS OF WEANLING PIGS FED DIETS SUPPLEMENTED WITH CHLOROACETIC ACID

N. AMAECHI¹ and U. P. NJOKU²

¹Department of Veterinary Microbiology and Parasitology, Michael Okpara University of Agriculture, Umudike, Nigeria

²College of Animal Science and Animal Production, Michael Okpara University of Agriculture Umudike, Nigeria

*E-mail: ndubueze65@gmail.com

ABSTRACT: This study investigated the effect of chloroacetic acid on growth performance and haematological parameters of weanling pigs. Thirty-six cross-bred weanling pigs (Landrace × Duroc) were allotted randomly to four treatment groups, with three replicates of three weanling pigs in each group. Control (T₁) weanling pigs were given a standard basal diet; Treatment 2, 3 and 4 were diets of 0.3, 0.6 and 0.9 percents levels of inclusion of chloroacetic acid respectively. After six weeks, blood and intestinal samples were collected from one animal per replicate. Data on feed intake and weight gain were collected daily. Results showed that chloroacetic acid did improve the animal growth performance. There was a decrease in pH. There was significant differences ($P < 0.05$) on white blood cell and mean corpuscular haemoglobin across the treatment. There was no significant difference ($P < 0.05$) across the treatments on pack cell volume and red blood cell count. This study showed that chloroacetic acid influenced some haematological parameters, decreased the pH of the gastrointestinal tract of the animals. Further studies will be needed to better understand the mechanisms underlying the effects observed when chloroacetic acid is fed to weanling pigs.

Key words: Nitrogen Chloroacetic Acid, Growth Performance, Haematological Parameters Weanling Pigs.

INTRODUCTION

Weaning in piglets is a crucial stage because the pigs are exposed to nutritional, environmental and social stresses resulting to low weight gain, nutrient malabsorption and increased occurrence of diarrhoea (Barnett et al., 1989; Boundry et al., 2004; Hedermann and Jensen, 2004). Antibiotics have been widely used to limit the impact of the post-weaning period on animal health. Nevertheless, antibiotic fed to farm animals may be responsible for the spreading of bacteria that are resistant to such antimicrobials (Bager et al., 1997; Philips et al., 2004). This led to the prohibition of the use of antibiotics as a growth promoter. The adjustments following the withdrawal of these products in animal production have been difficult at times and many replacement solutions have been proposed, more or less successfully by the feed additive industry. Organic acids are important approach that have potential to improve performance in animals (Patterson and Burkholder, 2003; Ricke, 2003) and also provide people with healthy and nutritious animal products (Ricke, 2003).

Chloroacetic acid is an organic acid that has been reported to be beneficial to weanling pigs helping them to overcome problems occurring during the post weaning period (Tsiloyiannis et al., 2001), and improved animal growth performances (Partenen and Mroz, 1999). Blood is important in the maintenance of physiological equilibrium in the body (Wilson and Mead, 1987). However, this equilibrium may be disturbed due to certain nutritional, environmental, physiological and pathological conditions. The knowledge of haematological values is useful in diagnosing various pathological and metabolic disorders, which can adversely affect the productivity and reproductive performance of weanling pigs hence, resulting in great economic losses to pig farmers (Okoli et al., 2008). The current research investigated the response of weanling pigs fed chloroacetic acid at various levels of inclusion in the diet on their growth performance and haematological parameters.

MATERIALS AND METHODS

Animals and experimental design

A total of 36 crossbred pigs (landrace × Duroc) were weaned at 35 days and transported to Piggery Unit of Michael Okpara University of Agriculture, Umudike, Nigeria. They were housed in individual pen (50×90cm) with

ORIGINAL ARTICLE

free access to feed and drinking water for 42 days. The experimental design was complete randomized design (CRD). Data obtained were subjected to analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test as described by Steel and Torrie (1980). The criteria for significance were a probability of 0.05. After a 7 days adaptation period during which all piglets received the same based diet, the pigs (8 ± 2 kg of body weight) were divided into 4 groups (9 animals per group) that were homogenous for weight and sex. The pigs received 1 of 4 diet treatments, consisting of the base diet with (a) no addition (control diet), or with the addition of chloroacetic acid at (b) 0.3%, (c) 0.6% and (d) 0.9%. All diets were formulated to provide the same amount of energy, protein, essential amino acids, calcium and phosphorous. Feed and water were provided on an *ad libitum* basis. Composition of the experimental diets is reported in Table 1.

Table 1 - Percentage composition of experimental diets

Ingredients	Experimental diets			
	CHLA 0.0%	CHLA 0.3%	CHLA 0.6%	CHLA 0.9%
Maize	25.00	25.00	25.00	25.00
Maize offal	10.00	10.00	10.00	10.00
Brewers dry grain	15.00	15.00	15.00	15.00
Groundnut cake	8.00	8.00	8.00	8.00
Palm kernel cake	6.00	6.00	6.00	6.00
Wheat offal	27.3	27.3	27.3	27.3
Soya meal	5.00	5.00	5.00	5.00
Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Bone meal	3.0	3.0	3.0	3.0
Salts	0.25	0.25	0.25	0.25
Vit (mineral premix)	0.25	0.25	0.25	0.25
Total composition	100	100	100	100
Calculated nutrient content				
Crude protein %	19.521	19.521	19.521	19.521
ME (Kcal/kg)	2432.71	2432.71	2432.71	2432.71
Chloroacetic acid	0%	0.3%	0.6%	0.9%

Data Collection

pH determination: To determine the pH, 10g of gut content from stomach, duodenum, jejunum, caecum and rectum were collected aseptically on 90ml sterilized physiological saline (1:10 dilution) Al-Natour and Alshwabkeh, 2005) and pH were determined.

Blood sample: At the end of the experiment, three pigs were randomly selected per treatment group and bled from a punctured jugular vein. The samples for haematological analysis were collected in bottles containing ethylene diamine tetracetic acid (EDTA) at 1.5 mg/ml of blood as anti coagulant for the determination of haematological indices of interest such as red blood cell (RBC), white blood cell (WBC) haemoglobin and packed cell volume (PCV). Values obtained were used to calculate the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) as described by (Dacie and Lewis, 1991).

Growth performance: The initial weights of the weanling pigs were taken at the beginning of the experiment. Weight gain was obtained by subtracting the initial live weight from final live weight. Data on feed intake were determined by difference between the quantity offered and quantity left over each day. Feed conversion ratio was obtained by dividing feed intake by weight gain.

RESULTS AND DISCUSSION

Effects of haematological values

The haematological values of weanling pigs fed different levels of chloroacetic acid are shown in Table 2. CHLA 0.0% had the highest value of WBC count followed by CHLA 0.9% and CHLA 0.3% while CHLA 0.6% gave the lowest value which were significant ($P < 0.05$). Okoli et al. (2008) reported a normal range of 3.00 to 4.20×10^3 for pigs. The higher value in CHLA 0.0% (5.20) may be as a result of the infection, while the normal value in CHLA 0.3% and CHLA 0.9% and lower value in CHLA 0.6% of WBC indicates lower level or absence of infection which may be due to the effect of chloroacetic acid acting as an antibiotic in the body. This result confirmed those obtained by Woldenden et al. (2007) and Abd El-Hakim et al. (2009) who concluded that organic acids could be used in animals, not only as a growth promoter but also as a meaningful tool of controlling intrinsic pathogenic bacteria. The red blood cell count values indicated that there were significant difference across the treatments ($P < 0.05$), which is in consonant with Akanno et al. (2008). For packed cell volume, CHLA 0.0% gave the highest value (34%), followed by CHLA 0.9% (33%) and CHLA 0.3% (32%) while CHLA 0.6% gave the lowest value (15%). CHLA 0.0%, 0.3% and CHLA 0.9% fell within the normal range (30-50%) as reported by Okoli et al., (2008). For haemoglobin concentration, CHLA 0.0%, 0.3% and CHLA 0.9% fell within the normal range (10-16g/dL) ad reported by Okoli et al. (2008). There were no significant differences across the haemoglobin concentrations. Thus Okoli et al. (2008)

reported a normal range of mean corpuscular haemoglobin concentration to be (30-36%) in which all the treatments fell within the normal range. For the control the MCHC falls below the treatments values, this indicates that chloroacetic acid improved the MCHC. There was a significant difference on mean corpuscular haemoglobin (MCH) across the treatments ($P < 0.05$). According to Okoli et al. (2008), CHLA 0.3% and CHLA 0.9% fell within the normal range; CHLA 0.0% fell above the normal range, while CHLA 0.6% fell below the normal range. Significant difference also existed on mean cell volume (MCV) among the treatments ($P < 0.05$).

Table 2 - Haematological values of weanling pigs fed chloroacetic acid at different levels

Parameters	Treatments				SEM
	CHLA 0.0%	CHLA 0.3%	CHLA 0.6%	CHLA 0.9%	
WBC ($\times 10^3$ /ml)	5.20 ^a	3.10 ^c	1.70 ^d	3.90 ^b	0.08
RBC ($\times 10^6$ /ml)	4.30 ^a	4.70 ^a	4.20 ^a	4.80 ^a	0.21
PCV (%)	34.00 ^a	32.00 ^a	15.00 ^b	33.00 ^a	2.35
Hb (g/dL)	11.20 ^a	10.80 ^b	7.01 ^e	11.07 ^a	0.06
MCHC (%)	32.94 ^c	33.75 ^a	33.33 ^b	33.33 ^b	0.01
MCH (pg)	26.05 ^a	22.98 ^b	16.66 ^d	22.92 ^c	0.01
MCV (μm^2)	79.67 ^a	68.09 ^b	50.00 ^c	68.75 ^b	1.45

Foot note: a,b,c,d- means in the row with different superscripts are significantly different from one another ($P < 0.05$). SEM = standard error of the mean.

Growth performance

The growth performance of weanling pigs fed chloroacetic acid at different levels is shown in Table 3. There were significant differences between the control and the treatments on final weight and average daily feed intake. This is in agreement with the report of Piva et al. (2002 b) who reported that adding chloroacetic acid to the diet of weanling pigs improve their appetite and have positive effect on weight gain. The production results with respect to daily weight gain and feed conversion ratio was superior in group fed chloroacetic acid as compared to those obtained in the control group (Russel and Diez-Gonzalez, 1998).

Table 3 - Growth performance of weanling pigs fed different levels of chloroacetic acid

Parameters	Treatments				SEM
	CHLA 0.0%	CHLA 0.3%	CHLA 0.6%	CHLA 0.9%	
Initial weight (g/animal)	6.70	5.93	6.57	6.47	0.94
Final weight (g/animal)	11.43	11.53	12.47	12.53	0.74
ADFI (g/animal/day)	0.81	0.83	0.81	0.81	0.01
ADWG (g/animal/day)	0.05 ^a	0.14 ^b	0.13 ^b	0.12 ^b	0.01
Feed conversion ratio	5.81	6.08	6.13	6.19	0.37

^{a,b} means in the row with different superscript are significantly different from one another ($P < 0.05$). SEM= standard error of the mean; ADFI= average daily feed intake; ADWG= average daily weight gain

There was no significant difference ($P > 0.05$) on feed conversion ratio (FCR) across the treatments. There was a significant difference ($P < 0.05$) between the CHLA 0.0% and other treatments. This implied that chloroacetic acid has slight effect on the daily weight gain of the pigs. This is in line with the report of Piva et al. (2002a). There was an increase in FCR from CHLA 0.3% to CHLA 0.9%. This showed that an increase in dietary inclusion of chloroacetic acid increases the FRC. At the same time, feeding high doses of organic acids may result in reduced feed intake and poor growth performance because of reduced feed acceptance.

Table 4 - pH values of weanling pigs fed chloroacetic acid at different levels

Parameters	Treatments			
	CHLA 0.0%	CHLA 0.3%	CHLA 0.6%	CHLA 0.9%
Stomach	5.37 ^c	4.50 ^c	4.33 ^c	4.53 ^c
Duodenum	5.28 ^c	4.48 ^c	4.50 ^c	4.50 ^c
Jejunum	6.37 ^b	5.47 ^b	5.47 ^b	5.63 ^b
Caecum	7.22 ^a	6.20 ^a	6.40 ^a	6.37 ^a
Rectum	7.17 ^a	6.01 ^a	6.17 ^a	6.33 ^a
SEM	0.25	0.20	0.23	0.22

^{a,b,c} means in the same column with different superscript are significantly different ($P < 0.05$)

Table 4 showed the pH of chyme from the gastro-intestinal tracts of weanling pigs fed different levels of chloroacetic acid. The pH of stomach, duodenum, jejunum, caecum and rectum varied significantly among all the treatments ($P < 0.05$). In caecum and rectum, the highest pH values were recorded (6.32 and 6.17 averages, respectively) because appreciable amount of bacteria fermentation takes place there, thereby increased the alkalinity. Lowest pH value was recorded in the stomach, duodenum and jejunum (4.45, 4.49, and 5.52 average, respectively) because undissociated organic acid crossed mucous membrane and were absorbed in the small intestine. This finding is in accordance to Piva et al. (2002 a,b). This will cause reduction of the pH in the small



intestine. But due to the presence of HCL and chloroacetic acid in the stomach, there was reduction in the pH of the stomach (Dibner and Butin, 2002).

CONCLUSION

This study showed that weanling pigs that received chloroacetic acid at 0.3 and 0.9 percent levels of inclusion gave better result on haematological parameters, increased the pH of the stomach, duodenum and jejunum. When fed to pigs after weaning, chloroacetic acid improved final weight and feed conversion ratio numerically. These results suggest that chloroacetic acid is worthy of further investigation as a potential alternative to antibiotics to improve growth performance and haematological values of pigs in the post weaned phase.

REFERENCES

- Abd El-Hakim AS, Cherian G and Ali MN (2009). Use of organic acids, herbs and their combination to improve the utilization of commercial low protein broiler diets. *International Journal of Poultry Science* 8(1): 14-20.
- Akanno EC, Aladi NO, Okeudo NJ and Okoli IC (2008). Reproductive and haematological characteristics of the Nigerian indigenous and large white pigs in a humid tropical environment *Journal of Animal and Veterinary Advances*. 3: 17-23.
- Al-Natour MQ and Alshawabkeh KM (2005). Using varying levels of formic acid to limit growth of *salmonella gallinarium* in contaminated broiler feed. *Asian Australian Journal of Animal Sciences* 18: 390-395.
- Bager F, Madsen M, Christensen J and Aarestrup FM (1997). Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus Faecium* on Danish poultry and pig farms. *Preventive Veterinary Medicine* 31: 95-112.
- Barnett KL, Kornegay ET, Risely CR, Lindemann MD and Shurin GG (1989). Characterization of creep feed consumption and its subsequent effect on immune response, scouring index and performance of weanling pigs. *Journal of Animal Science* 67: 2698-2708.
- Boundry G, Person V, Huerou-luron I and Lalles TP (2004). Weaning induces both transient and long lasting modification of absorption, secretary and barrier properties of piglet intestine. *Journal of Nutrition* 134: 2256-2262.
- Decie JV and Lewis SM (1991). *Practical haematology* (Revised edition) London; Lonelon group Limited. Pp 21-68.
- Dibner JJ and Butin P (2002). Use of organic acid as a model to study the impact of gut micro flora on nutrition and metabolism. *Journal of Applied Poultry Research* 11: 453-463.
- Hedermann MS and Jensen BB (2004). Variations in enzyme activity in stomach and pancreatic tissue and digester in piglets around weaning. *Archival of Animal Nutrition* 58: 47-59.
- Okoli IC, Aladi NO, Okeudo NJ and Akanno E (2008). Reproductive and haematological characteristics of the Nigerian indigenous and large white pigs in a humid tropical environment. *Journal of Animal and Veterinary Advances* 3: 17-23.
- Partenen KH and Mroz Z (1999) Organic acids for performance enhancement in pig diets. *Nutrition Research Reviews* 12: 1-30.
- Patterson JA and Burkholder KM (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science* 82: 627-631.
- Philips I, Casewell M, Cox T, De-Groot B and Waddel J (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy* 53: 28-52.
- Piva A, Casadei G and Biagi G (2002)a. An organic acid blend can modulate swine intestinal fermentation and reduce microbial proteolysis. *Canadian Journal of Animal Science* 82: 527-532.
- Piva A, Morlaichini M, Casadei G, Gatta PP, Biagi G and Prandini A (2002)b. Sodium butyrate improves growth performance of weaned piglets during the first period after weaning. *Italian journal of Animal Science* 1: 35-41.
- Ricke SC (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science* 82: 627-639.
- Russel JB and Diez-Gonzalez F (1998). The effects of fermentation acids on bacterial growth. *Advance Microbiology and Physiology* 39: 205-234.
- Steel RGD and Torrie JH (1980). *Principles and procedures of statistic. A biometrical approach*. 2nd edition. New York.
- Tsiloyiannis VK, Kyriakis SC, Vlemmas J and Sarris K (2001). The effect of organic acids on the control of porcine post-weaning diarrhea. *Research in Veterinary Science* 70: 287-293.
- Wilson MA and Mead EA (1987). Blood profile as a guide to nutritional status in animals. *British society of animal production* 33: 155-162.
- Woldenden AD, Vicente JL, Higgins JP and Tellez G (2007). Effect of organic acids and probiotics on *salmonella enteritidis* infection in broiler chickens. *International Journal of Poultry Science* 6: 403-405.



EFFECT OF DIETARY LEVELS OF SPEARMINT (*Mentha spicata*) ON BROILER CHICKS PERFORMANCE

E.O. AMASAIB^{1*}, B.H. ABD ELRAHMAN¹, A.A. ABDELHAMEED², B.A. ATTA ELMNAN and A.G. MAHALA¹

¹Department of Animal Nutrition, Faculty of Animal Production, University of Khartoum, Sudan

²Department of Animal Production, Faculty of Agricultural Science, University of Dongola, Sudan

*E-mail: samaniamasaib@gmail.com

ABSTRACT: This study was conducted to determine the effect of addition different levels of spearmint (*Mentha spicata*) on broiler chick's performance. One hundred and twenty eight day old unsexed (Cobb) broiler chicks were used in this experiment. Birds were distributed randomly into 16 pens (8/pen) as replicates, in a complete randomized design. The experimental diets were formulated with four levels of spearmint (*Mentha spicata*) of 0, 1, 1.5 and 2%. Feed and water were freely accessed. Feed intake, body weight gain and feed conversion ratio were weekly recorded and Mortality rate was recorded throughout the experiment. At the end of the experimental period, four birds from each treatment were randomly selected, weighed and slaughtered for determination of carcass weight and dressing percentage. Average feed intakes obtained from the experiment were 2680.20, 2679.11, 2708.55 and 2692.57 for diets 0, 1, 1.5 and 2%, respectively. However, the body weight gains for the treatments were 1481.63, 1512.81, 1519.57 and 1519.63, 0, 1, 1.5 and 2, respectively. Feed conversion ratios for treatments were found to be 1.92, 1.94, 1.92 and 1.99 respectively. Dressing percentage were 73.12, 74.17, 73.08 and 73.47 respectively. The results indicated that the supplementation of different levels of spearmint to the diets of broiler improved feed intake and body weight gain.

Key words: Spearmint (*Menthaspicata*), Broiler, Performance

ORIGINAL ARTICLE

INTRODUCTION

Organic poultry is relatively a new expression, which is widely spread in the developed countries and may be expand to the developing ones. In such kind of poultry production, farmers are not allowed to use chemical compounds at all or in a very low level for sake of costumers, instead they use alternatives like organic acids, probiotics, and medicinal plants, Mansoub (2011). Herbs and spices were used in poultry diets as feed additive non nutritive substance. In the presence of these substances the nourishing value of the ration are primarily included to improve the efficiency of the birds' growth, prevent disease and improve feed utilization. Herbs contain active substance that can improve digestion and metabolism (Sabra and Metha, 1990).

Mentha (Mint) mints are aromatic, almost exclusively perennial, rarely annual herbs that are widely distributed and can be found in many environments (Brickell, 2002). All mints prefer and thrive in cool moist spots in partial shade. They are fast growing, extending their reach along surfaces through a network of runners. Mint essential oil and menthol are extensively used as flavorings in breath fresheners, drinks, antiseptic mouth rinses, toothpaste, chewing gum, desserts and candies. The main medicinal action of the leaves and flowers of the mint depends on the abundant volatile oil, which has been found to contain a hydrocarbon, thymol and higher oxygenated compounds. It yields its virtues to boiling water, but particularly to alcohol. Steams are antispasmodic, choleric and carminative (Galib, et al. 2010). Mint is usually taken after a meal for its ability to reduce indigestion and colonic spasms by reducing the gastrocholic reflux (Spirling and Daniels, 2001).

The objective of the present study is to estimate the effect of feeding different levels of dietary spearmint (*Mentha spicata*) on broiler chick's performance.

MATERIAL AND METHOD

A total of 128 one-day old unsexed commercial broiler chicks (Cobb), were used in this study. Chicks were weighed and randomly divided into 4 groups of 32 chicks. Each group was further subdivided into four replicates with 8 chicks' per replicate. The initial weight of chicks ranged between 43.4 to 47.5g.

The First 7 days from the experiment was used as adaptation period, all chicks were fed the basal diet and assigned as untreated control. The experimental diets were formulated from basal diets (Table 1). And a graded levels of spearmint were added to the basal diet at a rate of (0, 1, 1.5 and 2%), and refers to as diet A, B, C and D respectively.

The proximate composition of spearmint was performed according to AOAC (1982) and was illustrated in Table 2. The average live body weights, body weight gains, feed intake and feed conversion ratio were measured on a weekly basis. Mortality rate for each treatment was recorded. Birds were slaughtered by cutting the throat and jugular vein using a sharp knife near the first vertebra. 4 birds from each replicate were selected randomly, slaughtered and the internal organs were dissected out. The chicks were weighted before and after slaughtering to determine hot weight and carcass weight. The dressing percentage was determined by expressing hot carcass weight to live weight. Completely Randomize Design was used in the experiment. The data generated from the experiment was subjected to Analysis of variance according to the SPSS using computer program. Duncan's multiple tests were used to assess significance of differences between treatment means.

Table 1 - Ingredient composition of experimental diets as percent (%)

Ingredient	Spearmint level %			
	(0)	(1)	(1.5)	(2)
Sorghum	63.79	62.98	62.35	61.70
G.N.M.	16.36	16.47	16.47	16.47
S.M.	13.50	13.00	13.00	13.00
Super concentrate	5.00	5.00	5.00	5.00
Spearmint	0.00	1.00	1.50	2.00
Lime stone	1.00	0.96	0.95	0.95
NaCl	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Oil	0.00	0.24	0.38	0.53
Total	100	100	100	100
Calculated nutrient content				
Crude protein (%)	22.78	22.71	22.72	22.73
Metabolizable energy (kcal/kg)	3110.36	3110.69	3110.31	3110.13
Calcium (%)	1.135	1.110	1.106	1.105
Total phosphorus (%)	0.665	0.658	0.656	0.654
Lysine (%)	1.119	1.114	1.113	1.111
Methione (%)	0.519	0.512	0.511	0.51
Crude fiber (%)	4.376	4.326	4.311	4.294
Crude protein (%)	23.26	23.34	23.49	23.52
Ether extract (%)	7.71	7.91	7.02	7.11
Ash (%)	6.97	6.09	6.94	6.56

G.N.M = ground nut cake meal S.M = sesame cake meal

Table 2 - Proximate analysis (%) of spearmint

Compound	Dry spearmint
DM	94.06
CP	19.25
EE	2.10
CF	19.57
Ash	12.82
NFE	40.32
ME/Kcal/kg)	1775

Metabolizable energy values of spearmint were calculated using the following equation according to Lodhi et al. (1976). $ME = 1.549 + 0.0102 CP + 0.0275 EE + 0.0148 NFE - 0.032 Cf$. ME for spearmint were 1775 Kcal/kg

RESULTS AND DISCUSSION

Feed intake

The effect of feeding graded levels of spearmint (*Mentha spicata*) on weekly feed intake is presented in Table 4. The results revealed that the dietary treatment had no significant effect ($P < 0.05$) on feed intake. The highest feed intake was obtained by the birds fed 1% spearmint during second and third week. The increment in feed intake which was illustrated in this study may be due to the flavor effect of spearmint (Deyoe et al., 1962). The insignificant effect of addition of spearmint to the basal diet may be due to the fact that, the diets were isocaloric and it is expected that the feed consumption could be similar (Scott et al., 1982), or may be due to the similar environmental during this period.

Body weight

The results of body weight gain are given in Table 5. The data is showing weekly body weight gain as affected by supplementation of spearmint. Birds fed 1% spearmint in the second and third week were grown better than

those fed higher levels of spearmint (1.5%, 2%). The body weight gain was not significantly ($P>0.05$) affected by addition of spearmint. These results were in the line of (Galib et al., 2010), who found insignificant effect of addition of peppermint on broiler body weight, but with improving performance compared to the control. Same results were noted by (Demir et al., 2008) concerning the effect of spearmint on broiler body weight.

Feed conversion ratio (FCR)

The data for feed conversion ratio is illustrated in Table 6. The results showed the effect of spearmint on feed FCR which was found to be insignificant in the first five weeks of age, but it is significantly affected by addition of spearmint in the sixth week ($P<0.05$), with the ranking to be as follows (1.84, 1.89, 1.94 and 2.04) for diet 1.5, 2, 1 and A. This may be due to change in environment during this week and increasing of bird's age.

Table 3 - Feed intake of boiler chicks (g/bird/week) as affected by addition of spearmint (*Mentha spicata*)

Weeks	Spearmint level %				± SEM
	(0)	(1)	(1.5)	(2)	
1	59.00 ^a	59.47 ^a	57.00 ^a	66.13 ^a	4.36
2	236.60 ^a	245.94 ^a	234.63 ^a	229.50 ^a	7.17
3	410.50 ^a	416.88 ^a	402.35 ^a	399.72 ^a	15.47
4	569.66 ^a	566.47 ^a	566.97 ^a	566.91 ^a	11.96
5	683.66 ^a	666.60 ^a	679.59 ^a	679.38 ^a	18.07
6	711.53 ^a	728.75 ^a	765.92 ^a	750.91 ^a	25.90

* Values are means of 4 replicate of 8 birds. * SEM = Standard error of the means. *Values with in rows with the same superscript are not statistically different ($P< 0.05$)

Table 4 - Body weight gain of boiler chicks (g/bird/week) as affected by addition of spearmint (*Mentha spicata*)

Weeks	Spearmint level %				± SEM
	(0)	(1)	(1.5)	(2)	
1	51.72 ^a	47.91 ^a	48.97 ^a	51.47 ^a	3.17
2	124.72 ^a	128.41 ^a	123.53 ^a	115.53 ^a	4.14
3	281.63 ^a	292.85 ^a	268.03 ^a	279.97 ^a	15.47
4	335.25 ^a	316.75 ^a	320.13 ^a	272.75 ^a	22.34
5	371.36 ^a	325.97 ^a	337.86 ^a	355.38 ^a	21.61
6	260.69 ^a	270.35 ^a	309.35 ^a	278.97 ^a	21.97

* Values are means of 4 replicates of 8 birds. * SEM = Standard error of the means. *Values with in rows with the same superscript are not statistically different ($P< 0.05$)

Table 5 - Feed conversion ratio as affected by addition of spearmint (*Mentha spicata*)

Weeks	Spearmint level %				± SEM
	(0)	(1)	(1.5)	(2)	
1	1.75 ^a	1.56 ^a	1.57 ^a	1.50 ^a	0.170
2	0.94 ^a	0.92 ^a	0.93 ^a	0.93 ^a	0.014
3	1.23 ^a	1.23 ^a	1.21 ^a	1.23 ^a	0.026
4	1.47 ^a	1.52 ^a	1.47 ^a	1.39 ^a	0.059
5	1.71 ^a	1.74 ^a	1.69 ^a	1.69 ^a	0.044
6	2.04 ^c	1.94 ^b	1.84 ^a	1.89 ^{ab}	0.029

* Values are means of 4 replicates of 8 birds. * SEM = Standard error of the means. *a,b,c values with in rows with different superscript differ significantly ($P< 0.05$)

Table 6 - Pre-slaughtering weight, carcass weight and dressing percentage of broiler chicks fed spearmint during 6 weeks

Weeks	Spearmint level %				± SEM
	(0)	(1)	(1.5)	(2)	
Pre-slaughtering weight (g)	1481.63 ^a	1525.81 ^a	1519.75 ^a	1519.63 ^a	42.2
Carcass weight (g)	1083.44 ^a	1131.75 ^a	1110.63 ^a	1116.53 ^a	38.79
Dressing percentage (%)	73.12 ^a	74.17 ^a	73.08 ^a	73.47 ^a	0.455

* SEM = Standard error of the means. *Values with in rows with the same superscript are not statistically different ($P< 0.05$)

Average pre-slaughtering weight (gm), carcass weight (gm) and dressing%

Dressing percentage of broiler chicks during experimental period is illustrated in Table 7. Four birds from each treatment were randomly selected and weighed before and after slaughtering to determined live weight and hot weight. The dressing weight to live weight. Dressing percentage for the four treatments found to be 73.12%, 74.17%, 73.08% and 73.47% respectively. The dressing percentage were not significantly ($P>0.05$) affected by addition of spearmint.



Effect of dietary spearmint (*Mentha spicata*) on overall performance

Data of overall feed intake, body weight gain, feed conversion ratio and dressing percentage are summarized in Table 8. Feed intake increased with increasing level of spearmint in the basal diet with the following ranking, Birds fed diet 1.5% obtained the highest feed intake (2708.55 g/chicks), followed by birds fed diet 2% (2692.57 g/chicks), A (2680.2 g/chicks) and the least feed intake was obtained by birds fed diet 1% (2679.11 g/chicks).

Table 7 - Effect of spearmint on overall performance throughout the experimental period (6 weeks)

Weeks	Spearmint level %				± SEM
	(0)	(1)	(1.5)	(2)	
Initial weight(g)	45.22 ^a	44.78 ^a	44.47 ^a	45.47 ^a	-
Final body weight (g)	1481.63 ^a	1512.81	1519.63 ^a	1519.63 ^a	42.20
Body weight gain (g)	1436.41 ^a	1468.03 ^a	1475.16 ^a	1474.16 ^a	38.79
Total feed intake (g)	2680.20 ^a	2679.11 ^a	2708.55 ^a	2692.57 ^a	63.81
FCR	1.92 ^a	1.94 ^a	1.92 ^a	1.99 ^a	0.042
Dressing (%)	73.12 ^a	74.17 ^a	73.08 ^a	73.47 ^a	0.455

* SEM = Standard error of the means
*Values with in rows with the same superscript are not statistically different (P< 0.05)

Body weight gain was also increased with increasing level of spearmint in the basal diet with the ranking found to be as follows, diet C recorded higher value for body weight gain when compared to diet A and diet B. It would be noted that as conclusion from the experiment, birds that fed diet (1.5% spearmint) were observed to have best performance in term of total body weight gain, total feed intake and economic value. These may attribute to the effect of some antimicrobial Components which may act as growth promoters (Al Ankari et al., 2004) and may be improvement of digestion and absorption of the nutrient (Brander, 1985). On the other hand Grieve (1981) and Chopra et al. (1992) referred the improvement in performance of the herb valued for its beneficial effect on the digestion.

CONCLUSION

Diet 1% spearmint improved feed intake and grown better during second and third week respectively. The study was emphasized that the best performance on birds fed diet 1.5% spearmint on body weight gain and total feed intake. Feed conversion ratio was similar for birds fed 1.5% spearmint with diet a control, while bird fed diet 2% spearmint was lowest.

REFERENCES

- AOAC (1980). Association of Official Analytical Chemists (official method of analysis). Washington, D.C, USA.
- Brander GC (1985). Growth promoters. In: Braner, G.C. Pough, D.M. and Bywater, R.J. (Ed), Veterinary Applied Pharmacology and Therapeutics. Biallieve Tindall, London, UK, pp., 430-445.
- Brickell Ch and Cole T (2002). The American Horticultural Society of plants and flowers. NewYork,NY,USA :DK Publishing, Inc. pp.605. ISBN 07894-8993-7.
- Chopra R, Nayar S and Chopra I (1992). Second Glossary of Indian Medicinal Plant. Publications and Information Directorate, New Delhi, India, P. 414.
- Demir E, Kilinc K, Yildirim Y, Dincer F and Eseceli H (2008). Comparative effects of mint, sage, thyme and flavomycin in wheat-based broiler diets. Archiva Zootechnica 11:3, 54-63.
- Deyoe CW, Davies RE, Krishnan R, Khaund R and Couch JR (1962). Studies on the taste performance of the chick. Poult. Sci., 41:781-784.
- Galib AM and Al-Kassie M (2010). The role of peppermint (*Mentha piperita*) on performance in broiler diets. Agric. Biol. J. N. Am., 2010, 1(5): 1009-1013.
- Grieve M (1981). A modern Herbal. Penquin, UK, p. 902. Halander, I., H. Alakomi, K. Latra-Kala, T. Matliila.
- Lodhi GN, Singh D and Lchopui JS (1976). Variation in nutrient content of feedingstuffs rich in protein and reassessment of chemical method for metabolizable energy estimation for poultry. J. Agric. Sci., 86: 293.
- MansoubN H (2011). Comparison of Effects of Using Nettle (*Urtica dioica*) and Probiotic on Performance and Serum Composition of Broiler Chickens. Global Veterinaria. 6(3):247-250
- Sabra KL and Metha TJ (1990). A comparative study on additive of livol (herbal growth promoter) and some chemical growth promoter in the diet of broiler chicken. Ind. J. Anim. Prod. Manage, 6: 115-118.
- Scott ML, Neshein and Young RJ (1982). Nutrition of chicken. Scott and Associated, eds., W.F. Humphrey Press, Inc., Ithacia, N.Y.
- Spirling LI, and Daneils IR (2001). Botanical perspectives on health peppermint: more than just an after - dinner mint. JR Soc Health. 121: 62 -63.



Online Journal of Animal and Feed Research (OJAFR)

Online ISSN: 2228-7701

Frequency: Bi-monthly

Current Volume: 3 (2013)

Current Issue: 4 (July)

Publisher: Science-line Publication

OJAFR is an open access peer-reviewed journal. All accepted articles are published bi-monthly in full text on the Internet. OJAFR publishes the results of original scientific researches, reviews and short communications, in all fields of animal and feed. It aims to improve livestock performance and better utilization of feed resources on animal's productions and related areas.

Field of Researches:

Nutrition (Ruminants and Non-Ruminants)
 Physiology and Functional Biology of Systems
 Behavior, Health and Welfare
 Farming Systems and Environment
 Agriculture: Agrobiology
 Nutritive value and utilization of feeds
 Mathematical models, analytical and experimental methods of feed evaluation
 Animal-feed interactions
 Dietary inputs
 Food Science and Technology: Food Safety and Health
 Product Quality, Human Health and Well-Being

OJAFR provides:

1. [Online submission portal](#)
2. Fast evaluation process
2. [Peer review by editors](#), who strive to give authors fair decisions and advice
3. [The highest standards](#) followed to improve your accepted manuscript
4. [OJAFR Publisher Item Identifier \(PII\)](#) and [Booklet](#) of each Issue
5. Scholarly Indexes to make your work more readable across the world
6. [Negligible editor fees](#) for registered members

Current Issue



Archive

[Call For Papers](#)
[Online Submission](#)
[Instructions for Author](#)
[MSword Template \(.doc\)](#)
[Declaration Form \(.doc\)](#)
[Application Form \(.doc\)](#)
[Join OJAFR Team](#)



Instructions for Author



Manuscripts as Original Research Paper, Short Communication, Case Report and Review or Mini-Reviews are invited for rapid peer-review publishing in [Online Journal of Animal and Feed Research \(OJAFR\)](#).

Papers can be in any relevant fields of Animal Sciences (Animal Nutrition, Physiology, Reproduction, Genetics and Breeding, Behavior, Health, Husbandry and its economic, Animal products and Veterinary medicines of domestic animals) and relative topics. The journal does encourage papers with emphasis on the nutritive value and utilization of feeds that is depended to methods of Improvement, Assessment, Conserving and Processing feeds, Agronomic and climatic factors, Metabolic, Production, Reproduction and Health responses to dietary inputs (e.g., Feeds, Feed Additives, Specific Feed Components, Mycotoxins). Also, Mathematical models relating directly to animal-feed interactions, Analytical and experimental methods for Feed Evaluation as well as Animal Production studies with a focus on Animal Nutrition that do have link to a feed (Food Science and Technology) are acceptable relative topics for OJAFR.

All manuscripts must be submitted in English and will be evaluated in a totally confidential and impartial way.

Submission of a manuscript to the OJAFR implies that:

1. Submitted work has not been previously published and is not being submitted for publication elsewhere;
2. All authors have approved the submission and have obtained permission for publish work.
3. Researchers have proper regard for conservation and animal welfare considerations. Attention is drawn to the '[Guidelines for the Treatment of Animals in Research and Teaching](#)'. Any possible adverse consequences of the work for populations or individual organisms must be weighed against the possible gains in knowledge and its practical applications.

The manuscript and other correspondence should be sent preferentially online by [OJAFR Manuscript Submission Portal](#) or by e-mails: editors@ojaf.ir or editorojaf@gmail.com.

PRESENTATION OF THE ARTICLE

Main Format: First page of the manuscripts must be properly identified by the title and the name(s) of the author(s). It should be typed in Times New Roman (font sizes: 17pt in capitalization for the title, 10pt for the section headings in the body of the text and the main text, double spaced, in A4 format with 2cm margins. All pages and lines of the main text should be numbered consecutively throughout the manuscript. The manuscript must be saved in a .doc format, (not .docx files). Abbreviations in the title are not allowed.

Article Sections Format:

Title should be a brief phrase describing the contents of the paper. The Title Page should include the author(s)'s full names and affiliations, the name of the corresponding author along with phone and e-mail information. Present address (es) of author(s) should appear as a footnote.

Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The abstract should be 150 to 300 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 **key words** that will provide indexing references should be listed.

Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and Methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the author(s)'s experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section.

Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Results and Discussion can be presented jointly if preferred.

Acknowledgments of persons, grants, funds, etc. should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph forms or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or PowerPoint before pasting in the Microsoft Word manuscript file. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

- *Examples (at the text):*

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001).

-- *Examples (at the end of manuscript, References section):*

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology. 7: 3535-3539.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Also, you can prepare your article according to Article Sample or Manuscript Template:

Download Article Sample:



Download Manuscript Template:



Review Process:

A double blind reviewing model is used by OJAFR. The manuscript is reviewed and edited by two reviewers selected by section editor or deputy SE of OJAFR, who are research workers specializing in the relevant field of study. Also, a reviewer result form is filled by reviewer to guide authors. One unfavorable review means that the paper will not be published. The costs of processing are covered by the authors or by the institutions from which the papers were sent, according to current rates of paper preparation and printing.

Manuscript Proof: After review and accepting paper, a final formatted proof as well as declaration form are sent to the corresponding author. The corrected proof should be returned within three days. The Editor reserves the right to forward the manuscript to press without submitting the final proof to the author. The Editor shall not be hold responsible for any mistakes shown in the final publication.

Article Processing Charge:

No Editing / Peer-Reviewing charges are required for publication of accepted articles in OJAFR. Article Processing Charge is a central mechanism for funding Open Access scholarly publishing like OJAFR which make their content available online to anyone and in doing so help solve the access challenges posed by subscription journals. Since [Science-line Journals](#) do not charge for access, we rely on other means of funding publication.

It depends on quality and acceptable format of submitted manuscripts.

Download declaration form

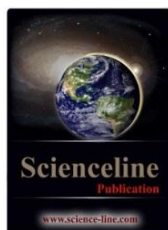


O J A F R

Online Journal of Animal and Feed Research (OJAFR)

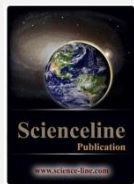
ISSN: 2228-7701

<http://www.ojafr.ir>



© 2013 Science-line Publishing Company

<http://www.science-line.com/index/>



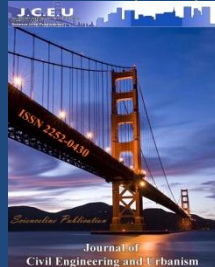
Welcome to Science Line (Online Publication)

The Science Line is a worldwide reporter of knowledge and research that takes aims to help scientists and researchers (especially from developing countries). The press is being run by a team of highly professionals from all corners of the world. The Recent Original Research Paper, Short Communication, Case Reports and Review or Mini-Review are invited for rapid peer-review publishing in our scientific journals that are listed below:

Online Journal of Animal and Feed Research (OJAFR)



Journal of Civil Engineering and Urbanism (JCEU)



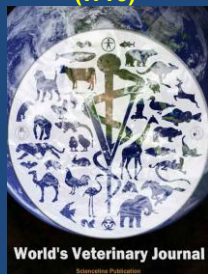
Journal of World's Poultry Research (JWPR)



Journal of Life Sciences and Biomedicine (JLSB)



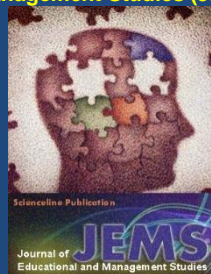
World's Veterinary Journal (WVJ)



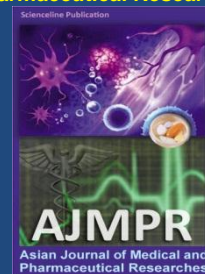
International Journal of Applied Sport Researches (IJASR)



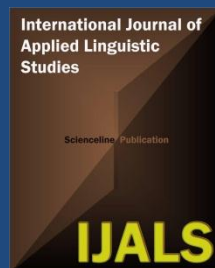
Journal of Educational and Management Studies (JEMS)



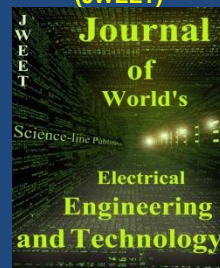
Asian Journal of Medical and Pharmaceutical Researches



International Journal of Applied Linguistic Studies (IJALS)



Journal of World's Electrical Engineering and Technology (JWEET)



Journal of Art and Architecture Studies (JAAS)



Scientific Journal of Mechanical and Industrial Engineering (SJMIE)



Science-line Publication Book Publishing Service

Science-line Publication provides publishing of books. Following is the basic steps to publish your manuscript/book.

1. Author(s) send manuscript to chief editor.
2. The primary review may take 1 to 4 weeks.
3. Review comments will be returned to author(s).
4. Peer-reviewers will be appointed in two weeks.
5. After peer-review, publishing contract will be applicable for author(s).
6. Payment is required for manuscripts contain with the treaty completed.
7. ISBN is assigned and ready for book publishing.
8. Final proof will be recommended from editor in chief.

We provide the best service in manuscript peer-review, editing, printing, transmitting and marketing evaluation. Welcome to publish your manuscript with Science-line Publication. For further information please visit homepage of Book Publishing Service

Science-line Journals are seeking qualified editors and reviewers with a Call for Papers notice. Willing to cooperate with the Science-line Press please contact us by email: scil.publishing@gmail.com