ICAR PUBLICATIONS

D. K. M. A.

636 (06) CODEN : IJLAA4 73 (10) : 1091-1194 (2003) II ISSN 0367-8318

The Indian Journal of Animal Sciences

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI

EDITORIAL BOARD

Dr V K Taneja (Chairman)
Deputy Director-General (Animal Sciences)
Indian Council of Agricultural Research, Krishi Bhawan, New Delhi 110 001

Dr A Ayyappan
DDG (Fisheries), Indian Council of Agricultural Research, Krishi Anusandhan Bhavan-II, Pusa, New Delhi 110 012

Dr J M Bujarbarua
Director, ICAR Research Complex for NEH Region, Umroi Road, Umiam, Bara Pani, Meghalaya 793 103

Shri A Chakravarty
Director, Directorate of Information and Publications of Agriculture, Krishi Anusandhan Bhavan-I, Pusa, New Delhi 110 012

Dr J S Dhillon
Formerly Dean (Veterinary Faculty) 167 H, Bhai Randhir Singh Nagar, Ludhiana (Punjab)

Dr S K Dwivedi
Director, National Research Centre on Equines, Hisar, Haryana 125 001

Dr R Kadirvel
Vice Chancellor, Tamil Nadu Animal Sciences and Veterinary University, Chennai, Tamil Nadu 600 007

Dr Amresh Kumar
Dean, Veterinary College, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttaranchal 263 145

R R Lokeshwar
Formerly Chief Editor (English), 5712, Vedavathy Block, Nandi Enclave, 2nd Cross, III Phase, 5th Block, Banashankar Stage 3, Bangalore 560 085

Dr K L Raheja
Dean, College of Animal Sciences, CCS Haryana Agricultural University, Hisar, Haryana 125 004

Dr M V Subba Rao
Dean, Faculty of Veterinary Science, ANG Ranga Agricultural University, Hyderabad, Andhra Pradesh 500 030

Dr P A Shankar
Director Instructions, College of Dairy Science, University of Agricultural Sciences, Hebbal, Bangalore, Karnataka 560 024

Dr N Sharma
Director, National Dairy Research Institute, Karnal, Haryana 132 001

Dr A T Sherikar
Vice Chancellor, Maharashtra Animal Science and Fishery University, Seminary Hills, Nagpur, Maharashtra 440 006

Dr M P Yadav
Director, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Assistant Editor : Aruna T Kumar

Incharge (English Editorial) : C S Vishvanath

Editorial Associate : J G Pandey

Chief Production Officer : Virender Kumar Bharti

Technical Officer : Anil Seth

Senior Artist : Narendra Bahadur

	Inland	Foreign
Single	Rs 75	UK £ 5, US $ 7
Annual Subscription	Rs 900	UK £ 60, US $ 84 (for Surface mail) UK £ 70, US $ 100 (for Air mail)

For any editorial enquiry please contact:

Aruna T Kumar, Ph: 011-25841004, 011-25734906 Extn. 612
E-mail: ijans@kab.delhi.nic.in

For subscription and advertisement please contact:

S K Joshi
Business Manager
Directorate of Information and Publications of Agriculture
Indian Council of Agricultural Research
Krishi Anusandhan Bhawan, New Delhi 110 012
Telefax: 011-25843657, E-mail: bmicar@kab.delhi.nic.in

• All disputes are subject to the exclusive jurisdiction of competent courts and forums in Delhi/New Delhi only • The Council does not assume any responsibility for opinions offered by the authors in the articles and no material in any form can be reproduced without permission of the Council • The Council is not responsible for any delay, whatsoever, in publication/delivery of the periodicals to the subscribers due to unforeseen circumstances or postal delay • Readers are recommended to make appropriate enquiries before sending money, incurring expenses or entering into commitments in relation to any advertisement appearing in this publication. The Council does not vouch for any claims made by the advertisers of products and services. The publisher and the editor of the publication shall not be held liable for any consequences in the event of such claims not being honoured by the advertisers.

THE INDIAN JOURNAL OF
ANIMAL SCIENCES

Previous Issue : Vol. 73, No. 09, pp. 955-1090

Vol. 73, No.10 October 2003

CONTENTS

ANIMAL HEALTH

Short communications

ANIMAL PRODUCTION

Short Communications

FISHERIES

Indian Journal of Animal Sciences 73 (10): 1093-1098, October 2003

A live attenuated vaccine for sheep-pox*

V BHANUPRAKASH[1], A R S MOORTHY[2], G KRISHNAPPA[3], R N SRINIVASA GOWDA[4] and B K INDRANI[5]

University of Agricultural Sciences, H A Farm (P.O), Hebbal, Bangalore, Karnataka 560 024 India

Received: 24 June 2002; Accepted: 30 May 2003

ABSTRACT

A live attenuated sheep-pox vaccine was prepared using Rumanian Fanar (RF) strain at 36th passage in secondary lamb testicular (SLT) cell system. The $TCID_{50}$ in SLT cells and reactive $dose_{50}$ (RD_{50}) in lambs were $10^{5.63}$ and $10^{7.44}$/ml respectively. The skin infective $dose_{50}$ (SID_{50}) of SPV challenge virus in lambs was 10^5/ml. The vaccine was innocuous in guinea pigs and mice; safe, potent and economical in SPV sero (antibody)-negative lambs. The vaccine was immunogenic in large flocks vaccinated at the field level, as measurred by glucose utilization test (GUT) and serum neutralization test (SNT) among the randomly selected sheep, for 6 months. The vaccine was found to be the best alternative to the conventional inactivated vaccines, and can be safely applied at the field level to eradicate the disease from the state and the country.

Key words: Eradication, Immunization, Live attenuated vaccine, Sheep, Sheep-pox virus, Vaccine

Sheep-pox, a highly contagious and devastating disease of sheep, occurs in Asia and Africa, with sporadic cases in European countries (Carn 1993). It has not been reported from the Americas and Australia and has been eradicated from most of the developed countries (Sathe 1931). The virus is transmitted by aerosol route, close contact and mechanically, biting flies also transmit the infection (Carn 1993).

Serve economic losses are due to high mortality in lambs, abortions and mastitis in ewes, skin condemnation, loss of wool and mutton in adult sheep besides, restricting the export potential of meat, wool and skin (Ramyar 1965).

The disease is endemic in India and several severe outbreaks have been reported regularly from almost all the states including Karnataka. It is a major sheep rearing state with more than 7.5 million sheep, which are mainly rared by small and marginal farmers and for them it forms an important source of family income but lately it was cut down considerably due to sheep pox (Anon 1998-99).

*Part of Ph.D. thesis submitted by the first author to the University of Agricultural Sciences, Hebbal, Bangalore, Karnataka-560 024, India, in 2001.

Present address: [1]Scientist (Senior Scale), ERV and Clinical Virology Laboratory, Indian Veterinary Research Institute, Mukteshwar, Uttaranchal 263 138.

[2,5]Profcssor, Department of Veterinary Microbiology, Veterinary College.

[3]Director, Institute of Animal Health and Veterinary Biologicals Hebbal, Bangalore, Karnataka 560 024.

[4]Study Director and Assistant Manager, Rallis, Research Centre, Plot Nos 21 and 22, P.B.No. 5813, Peenya II phase, Bangalore, Karnataka 560 024.

Presently, inactivated vaccine is used, which is besotted with problems like short duration of immunity, difficulty in large-scale production, handling, transportation and storage (Pandey and Nanda 1988).

The aim of this work was to prepare and study the efficacy of a live attenuated sheep-pox vaccine at the laboratory and field levels made from SPV-RF strain, which will be useful in future for the prevention of sheep-pox with a long-term objective of eradication in the state and so from the country.

MATERIALS AND METHODS

Bannur crossbreed lambs (20), 3- to 5-month-old, SPV sero (antibody)-negative were used. Secondary lamb testicular cell system (SLT) prepared from primary lamb testicular cells (PLT) was used for the propagation of SPV (Mateva *et al.* 1974). The live attenuated sheep-pox virus-Rumanian Fanar (SPV-RF) at 35th and 36th passage was available at the Institute of Animal Health and Veterinary Biologicals (IAH & VB), Bangalore, Karnataka. The virus was propagated in SLT cells grown in Dulbecco's minimal essential medium (D-MEM) containing 10% neonatal calf serum. The virulent SPV challenge virus was procured from the Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu.

Vaccine

SLT cells were infected with SPV-RF and incubated at 37°C for 7 days. When the cytopathic effect (CPE) was 80%, the inoculated cells were frozen and thawed thrice. The viral suspension was clarified at 600×g for 10 min and the supernatant was tested for bacterial, mycoplasmal and fungal

contamination. SNT and AGPT confirmed the presence of SPV in the supernatant. The supernatant was mixed with an equal quantity of stabilizer (2.5% lactalbumin hydrolysate, 5% surcose in D-MEM, pH 7.6), distributed in 1ml aliquots, freeze-dried, labeled and stored at -40°C.

$TCID_{50}$ of vaccine

The freeze-dried vaccine was titrated in 20-well cell culture plates with SLT cell system (Pandey and Nanda 1988). Randomly selected freeze-dried vaccine vials (5), were each reconstituted with 1ml of maintenance medium, pooled and serial 10-fold dilutions were done in maintenance medium. Five wells were infected with 1ml of each of the viral dilutions and 5 uninfected wells served as cell controls. The plates were incubated at 37°C under 5% carbon dioxide for 7 days and examined daily for the appearance of CPE. Final results were recorded for the presence or absence of CPE respectively on day 7, and $TCID_{50}$ was calculated (Reed and Muench 1938).

Reactive $dose_{50}$ (RD_{50}) of the vaccine

SPV sero-negative male lambs (2), 3- to 5-month-old were used. The area over the abdomen was shaved, thoroughly washed with soap water followed by a wash with distilled water. Then the cleaned area was made moisture free. Randomly selected vaccine vials (5), each reconstituted with 1ml of PBS, pH 7.4 was pooled and serial 10-fold dilutions were done from 10^{-1} to 10^{-7}.

Vaccine virus (200 microlitres of 10^{-3} dilution) was applied intradermally into the centre of the square, to each of the 2 lambs begining at the thoracic end followed by 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions and a PBS control at the flank region. Five squares were used for each dilution. The experimental area was then covered with double-layered gauze cloth and secured well so as to prevent scratching and soiling. Temperature was recorded daily and the inoculated sites were examined for local reactions. Final reading was taken on the 10th day post inoculation and RD_{50} was determined (Reed and Muench 1938).

Propagation of challenge virus

SPV sero-negative female lambs (3), 3- to 5-month-old were used. Preparation of the abdominal area and markings were done as described earlier. The freeze-dried virus was reconstituted and 1 in 50 dilution in PBS (pH 7.4) was prepared. Eight sites, well apart, 4 on each side, were chosen for inoculation and at each site, 5-6ml of the viral suspension was inoculated subcutaneously and the experimental area was covered with 2-3 layers of sterile gauze cloth which was changed daily to avoid soiling and contamination of the inoculated sites. Temperature was recorded daily.

On day 7 the inoculated sites were examined for hyperemia and pendular appearance. After exsanguinations of the lambs, tissue and gelatinous material were collected from the inoculated sites under sterile ice-cold conditions. A 25% suspension was prepared by blending followed by addition of stabilizer. The suspension was strained through sterile gauze and filtered through 0.45μm filter, treated with gentamycin (30μg/ml), distributed in 1ml aliquots, freeze-dried and stored at –40°C.

Skin infective $dose_{50}$ (SID_{50}) of challenge virus

A 3-month-old, SPV sero-negative female lamb was used. The titration was done as described for RD_{50} titration of vaccine earlier and the final reading was taken on day 10-post inoculation and the SID_{50} was calculated (Reed and Muench 1938).

Experimental design

Innocuity test: The vaccine was tested for its innocuity in 2 guineapigs and 6 mice. Each of the guineapigs was inoculated intradermally and intramuscularly with 0.5ml of the vaccine (10^3TCID_{50}/dose) and each of the mice was inoculated intraperitoneally with 0.2ml and observed daily for 14 days. The temperature of the guineapigs was recorded daily and the mice were observed for mortality (Pandey and Nanda 1988).

Safety test: SPV sero-negative lambs (2) were each inoculated with 10 field doses (1 field dose= 10^3TCID_{50}) of the vaccine contained in 1ml by subcutaneous route at the abaxial surface of the tail and observed daily for 14 days for the development of any untoward reactions (Pandey and Nanda 1988).

Potency test:Two groups, each with 4 SPV sero-negative lambs, were inoculated intradermally in the caudal fold, with 1 field dose (10^3TCID_{50}= group 1) and one-tenth of the field dose (10^2TCID_{50}= group 2), respectively, contained in 0.1ml. The lambs were observed daily for 14 days for local and general reactions. Body temperature of all the lambs including the controls was recorded daily (Pandey and Nanda 1988).

Challenge studies: Each group consisted of 4 lambs. All the vaccinated (group 2 and 1), in contact (group 3) and healthy control (group 4) lambs were challenged on 28th day-post vaccination with 10^4SID_{50} of the virulent SPV. The lambs were kept under observation for 14 days and the body temperature, local reactions and clinical symptoms, if any, were recorded daily and compared with the control groups (Pandey and Nanda 1988).

Field trials: Sheep (8655) at various organized and unorganized farms located at different agro-climate regions in Karnataka were vaccinated at an immunizing dose of 10^3TCID_{50}subcutaneously on the abaxial surface of the tail. Since challenge studies were not permitted at the field level, up to 10% of the blood and sera samples were collected from the vaccinated and control group on days 0,21,45,90 and 180-post vaccination to study the CMI and humoral responses respectively.

Assay of cell-mediated immunity (CMI): The CMI response was measured by glucose utilization test (GUT) (Awati 1992).

Blood was collected from vaccinated animals for sensitized lymphocytes and also from controls. Blood was collected aseptically from each lamb in a heparinized tube (20IU units/ml) and 0.2ml was cultured in 2ml RPMI-1640 (1x) medium in quintuplicates. Then 0.3ml of the vaccine with a tire of 10^5TCID_{50}/ml was added to each tube. The contents were mixed well kept at 37°C for 4 days with period agitation at 12-hr intervals. Similarly, 5 controls containing blood from unvaccinated lambs were maintained. Later, the tubes were centrifuged at 1500rpm for 10 min. The supernatant from the vaccinated and control lambs was pooled seperately. Then 0.2ml of the supernatant from each group was taken and diluted to 1 ml with distilled water. Phenol reagent 500 microlitres (5% in distilled water) and 5ml of concentrated H_2SO_4 was added to this mixture and allowed to stand for 10 min. The tubes were shaken well and placed in a water-bath for 15 min at 28°C. Distilled water served as the blank. The results were read in spectronic at 490nm.

Assay of humoral response: The humoral immune response was measured by SNT. Serial 2-fold dilutions of each serum were mixed with 10^2TCID_{50} of SPV-RF strain in 96-well microplates. The plates were held at 35°C for 1 hr and 10^5 SLT cells were then added to each well. The plates were sealed and incubated at 37°C for 7 days under 5%CO_2. The final reading was taken on day 7. The titre of each serum was expressed as the reciprocal of the highest dilution on the serum, which inhibited CPE. Neutralization index was calculated (Ramyar and Hessami 1970).

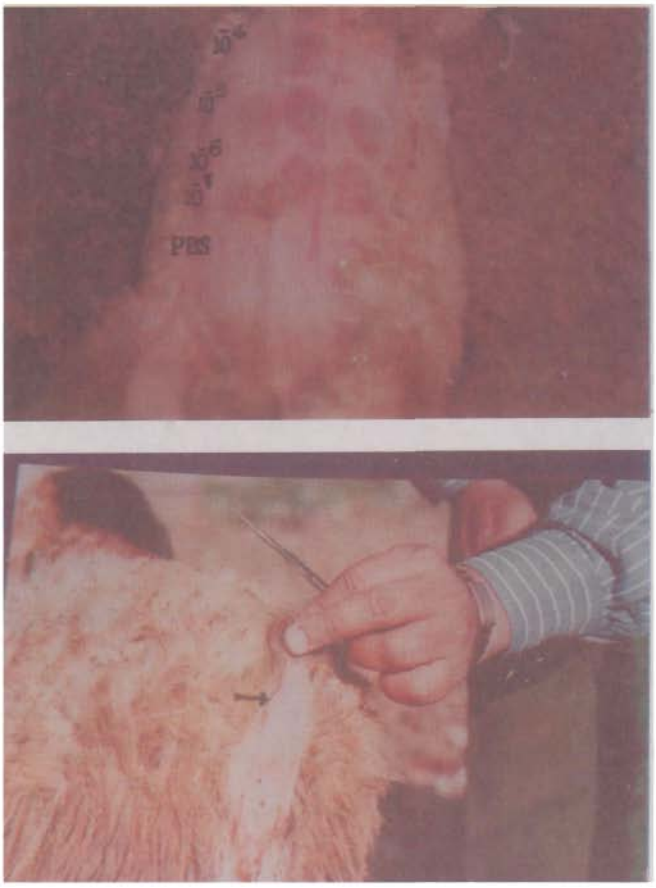
Statistics

The GUT and SN index values were analyzed by student's t-test and statistical significant differences are indicated by superscripts (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Virus titres

In *in-vivo* models (lambs), the appearance of takes at the sites of inoculation of SPV-RF during RD_{50} determination (Fig.1) coincided with the rise in body temperature (106.4°F) on seventh day and subsided to normal (102°F) on day 9. In this study, variations in virus titres were observed between *in vitro* ($10^{5.63}TCID_{50}$/ml in SLT cells) and *in vivo* ($10^{7.44}$ RD_{50}/ml in lambs) models. However, the $TCID_{50}$ titres were considered for vaccine dose fixation as done and observed by various workers with different strains of SPV (Jassim and Hawa 1981, Hegde 1988, Kalpana 1993). Thus, it appears that these were influenced by various viral factors (strain, pathogenicity, virulence, number of passages), cell systems (type, availability of receptors and fibroblasts) and animal factors (age, sex, breed, nutritional and immune status). As the titre vary from *in-vitro* to *in-vivo* tests, it was necessary to include both models for assaying the viral titres. Further, Ramesh (1980) reported it and we also noticed that viral titers were high in lamb testes and may be the SLT cell cultures were best suited for large-scale production of SPV.



Figs 1-2. **1.** Lamb exhibiting 'takes' at the sites of inoculation wih SPV-RF strain during RD_{50} titration **2.** Lamb exhibiting 'mild takes' on the abaxial surface of the tail after vaccination with SPV-RF strain on fifth day.

Propagation of challenge SPV

There was gradual increase in temperature in all the lambs inoculated with virulent strain of SPV 3 days after inoculation and the highest temperature was recorded on the seventh (105.5°F) and eighth (105.7°F) day post inoculation. The 'takes' started appearing on day 5-post inoculation and they became hyperemic and pendulous on days 6 and 7 due to tissue reactions and accumulation of fluids. The tissue material and fluids (100g/lamb) were collected after exsanguinations on ninth and tenth day. A 25% suspension in PBS was prepared, filtered, an equal volume of stabilizer added, distributed in 1ml aliquots and stored at –40°C for further use.

SID_{50} of virulent SPV

The inoculated lambs showed a gradual rise in body temperature (103.4°F) from day 4 onwards, reached a peak (106.0°F) on day 8 and receded to normal on day 13. The 'takes' appeared on day 5 after inoculation for 10^{-3} and 10^{-4} dilutions and on the day 6 for 10^{-5} dilution. The control sites

did not exhibit any 'takes'. The final reading was taken on day 10-post inoculation. The challenge SPV had a considerable titre (10^5 SID_{50}/ml) in *in-vivo* models as reported by Shome (1988) and this confirmed the pathogenic potential of virus to lambs.

Innocuity, safety and potency tests

Guineapigs and mice inoculated with SPV-RF vaccine remained healthy and normal during the observation period and hence, the vaccine was found innocuous in experimental animals as recorded by Pandey and Nanda (1988).

Both the lambs, used for safety testing of vaccine, showed gradual rise in body temperature attained an average peak of 106.7°F on day 5 post inoculation and subsided to normal on day 7. Both lambs showed distinct swellings at the sites of inoculation measuring about 0.5cm on day 5 post inoculation. The 'takes' were lential on day 5, attained a size of peanut on day 11 and subsided from day 12 onwards.There were neither local necrotic lesions nor generalization in both the lambs. 'Takes' were confined to the sites of inoculation and no other clinical signs were observed. This proved that the vaccine virus was safe and sufficiently attenuated for use as vaccine. Similarly, earlier workers used cell culture systems for attenuation of different strains of SPV (Martin *et al.* 1973, Jassim 1979, Ramesh 1980).

The 4 lambs each of group 1 and 2, vaccinated, showed a rise in body temperature (103.9-104.7°F) by day 7 and subsided to normal by day 8. The unvaccinated in-contact lambs (group 3) were normal during the entire observation period. The 'takes' at the site of vaccination appeared in both the groups 4-5 days post vaccination and were initially lential, later attained peanut size and subsided on day 8. The vaccinated lambs exhibited neither lesions nor clinical signs. The takes were very much confined to the site of vaccination

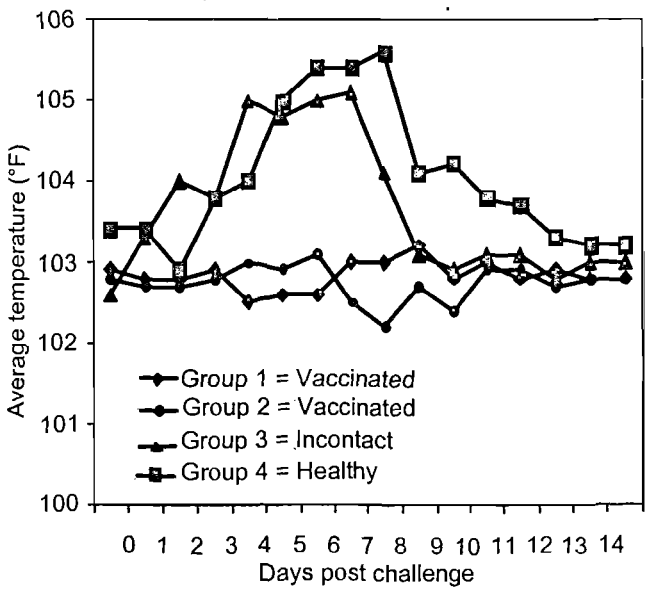


Fig 3. Average temperature reactions of live attenuated SPV-RF vaccinated and control lambs after challenge with virulent SPV

(Fig.2). The un-vaccinated in-contact lambs (group 3) did not exhibit any clinical signs or local reactions. In this study, the vaccine produced mild 'takes' confined to the sites of vaccination, without any untoward effects, which indicated complete attenuation of the virus. All the in-contact sero-negative lambs were normal which reflected non-shedding of virus from the vaccinates. However, back passage was not done in the present study. Jassim (1979), Ramesh (1980) and Awati (1992) opted for the abaxial surface of the tail for vaccination as in the present study, as it is advantageous. As compared to inactivated vaccines, live vaccines are more reliable and produce better immunity. Attenuation of virulent virus by various means is a popular method of production of live vaccines as it is more economical, dependable and reproducible (Shome 1988).

Challenge studies

Vaccinated lambs in each of the groups 1 and 2, did not show any thermal reactions after challenge with virulent SPV during the observation period, whereas, the in-contact (group 3) and healthy control lambs (group 4) exhibited severe thermal reactions (Fig.3), severe local reactions and other associated clinical signs of sheep-pox. Challenge study, the method of choice to assess the protection offered by SPV vaccines (Ramesh 1980, Awati 1992) was done at the laboratory level, and the vaccinated sheep withstood challenge but the controls succumbed.

Field trials

Among 8655 sheep vaccinated during field trials, only 165 sheep were closely monitored for 10 days after vaccination for temperature and local reactions. An average rise of 2°F in sheep vaccinated was observed. The 'takes' at the site of vaccination appeared after 2-4 days and subsided by day 8. The feeding behavior of the sheep was normal and no sheep pox specific signs was observed. As per the available literature, no field trails were conducted with such a large number of sheep situated in different agro-climate zones as was done in the present study to ascertain the efficacy of SPV-RF vaccine at the field level. No untoward effects or outbreaks were reported even after 6 months in the flocks vaccinated, although these were recorded in neighbouring unvaccinated flocks. This indicated that the vaccinated sheep were fully protected against natural outbreaks.

Rafyi and Chamsy (1956) reported the absorbance of the live SPV to gel and used it as vaccine and recorded strong immunity. But, in this study, also as reported by Yathinder (1984), the vaccine diluted in sterile cold PBS without the use of adjuvant was economical and advantageous.

CMI response

Since challenge studies at the field level are not permitted, periodic sero-monitoring was done to assess the CMI and humoral responses. There was a statistically significant difference ($P \leq 0.05$) in OD values by GUT between the control

and vaccinated group of sheep in the farms vaccinated for 180 days post inoculation (Table 1) indicating the persistence of CMI response, as reported by Kalpana (1993) with various strains of SPV as vaccine.

Table 1. Mean ± SE of OD (490nm) values of GUT of SPV-RF vaccinated and control sheep at farms- A and B

Types of samples	Mean±SE of OD (490 nm) values of GUT Days post vaccination				
	0 day	21 day	45 day	90 day	180 day
		Farm-A			
Vaccinated sheep	1.57^{a} ±0.016	1.34^{b} ±0.017	1.44^{c} ±0.049	1.45^{c} ±0.014	1.52^{c} ±0.026
(n)	(10)	(10)	(10)	(10)	(10)
Control sheep	1.58 ±0.025	1.55 ±0.00	1.58 ±0.025	1.58 ±0.025	1.75 ±0.050
(n)	(2)	(2)	(2)	(2)	(2)
		Farm-B			
	0 day	30 day	60 day		
Vaccinated sheep	1.6^{x} ±0.018	1.34^{y} ±0.037	1.29^{y} ±0.030		
(n)	(15)	(15)	(15)		
Control sheep	1.68 ±0.025	1.7 ±0.050	1.73 ±0.025		
(n)	(2)	(2)	(2)		

n, Number of samples; SE= standard error; the different superscripts indicate a significant difference between the mean values (P≤0.05).

Table 2. Mean ± SE of SN indices of vaccinated and control sheep at farms- A and B vaccinated with live attenuated SPV-RF vaccine

Types of samples	Mean±SE values of SN indices Days post vaccination				
	0 day	21 day	45 day	90 day	180 day
		Farm-A			
Vaccinated sheep	-	1.14^{a} ±0.067	2.03^{b} ±0.107	1.85^{b} ±0.075	2.32^{c} ±0.071
(n)	(10)	(10)	(10)	(10)	(10)
Control sheep	-	-	-	-	-
(n)	(2)	(2)	(2)	(2)	(2)
		Farm-B			
	0 day	30 day	60 day		
Vaccinated sheep		1.63^{x} ±0.027	2.18^{y} ±0.094		
(n)	(15)	(15)	(15)		
Control sheep					
(n)	(2)	(2)	(2)		

-, Negative, SE, standard error; n, number of samples. The different superscripts indicate a significant difference between the mean values (P≤0.05).

Humoral response

There was rise in neutralizing index (Table 2) from the day 21 post vaccination indicating the antigenicity and immunogenicity of the virus among the vaccinates in the 2 farms. The different mean values were statistically significant (P≤0.05). Rafyi and Chamsy (1956) and Shome (1988) indicated high levels of neutralizing antibodies on day 21 post vaccination. None of the vaccinated sheep got infected during the observation period of 200 days confirming the results of CMI and humoral responses.

We observed that the live attenuated SPV-RF vaccine at the 36th passage was innocuous, safe, immunogenic and protected against virulent challenge. The vaccine tested on a large population at the field level was efficient, effective, economical and mounted appropriate immune responses for the 6-month study period, projecting it as the best alternative to the conventional inactivated SPV vaccines. Additional feature of the vaccine is that more number of doses of effective vaccine could be made economically for field immunization based on both *in-vitro* and in *in-vivo* titers. Hence, with the use of this vaccine at the appropriate time, the entire sheep population can be easily vaccinated effectively at a reduced expense. If regular vaccinations are undertaken for 2-3 years, sheep-pox can be eradicated from the state as well as the country.

ACKNOWLEDGEMENTS

The author thanks the Director and the Joint Director, IVRI, and Dr R Hedge, Dr M M Kumar and Dr B Y Shridhar, IAH and VB, for the encouragement and technical support rendered by them.

REFERENCES

Anon. 1998-99. *Report on integrated sample survey for estimation of production of milk, egg, wool and meat for the year 1998-99.* Directorate of Animal Husbandry and Veterinary Services, Karnataka pp. 18.

Awati B. 1992. 'Safety and potency tests of Kenyan strain of sheep pox virus vaccine in goats'. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Carn V M. 1993. Control of capripox virus infections. *Vaccine* **11**(13): 1275-79.

Hedge R. 1988. 'Studies on antigenic analysis of Ranipet strain of sheep-pox virus'. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Jassim F A. 1979. 'Biological properties of Ranipet strain of sheep-pox virus'. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Jassim F A and Hawa L H. 1981. Adaptation and attenuation of local strain of sheep-pox virus in lamb testicle monolayers system. *Indian Veterinary Journal* **58**(10): 757-62.

Kalpana G. 1993. 'Evaluation of immunity conferred by Kenyan strain of capripox virus against goatpox infection and the

antigenic relationship between the two viruses'. M.V.Sc Thesis,University of Agricultural Sciences, Bangalore.

Martin W B, Ergain H and Koylu A. 1973. Tests in sheep of attenuated sheep pox vaccines. *Research in Veterinary Sciences* **14**: 53-61.

Mateva V, Jassim F A, Thompson J R and Al-Doori T M. 1974. The propagation of an attenuated sheep pox virus and its use as a vaccine. *Bulletin De Office L' Internationale des Epizooties* **81**: 329-39.

Pandey A K and Nanda Y P. 1988. *Laboratory manual for tissue culture sheep pox vaccine production.* IVRI, Izatnagar, U P, India.

Ramesh K G. 1980. 'Immunological studies on the Ranipet strain of sheep pox virus propagated in lamb testes cell culture'. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Rafyi A and Chamsy M H. 1956. Seven years' control of sheep pox in Iran with an absorbed tissue vaccine on aluminium gel. *British Veterinary Journal* **112**: 541-47.

Ramyar H. 1965. Studies on the immunogenic properties of tissue culture sheep pox virus. *Zentralblatt fur Veterinar Medizi* **12B**: 537-40.

Ramyar H and Hessami M. 1970. Studies on the duration of immunity conferred by a live modified sheep pox tissue culture virus vaccine. *Zentralblatt fur Veterinar Medizi* **17B**: 869-74.

Reed L J and Muench H. 1938. Simple method of estimating 50% end points. *American Journal of Hygiene* **27**: 493-97.

Sathe R G. 1931. Pox in sheep. *Indian Veterinary Journal* **8**: 118-21.

Shome B R. 1988. 'Comparative biochemical and immunological studies on polypeptides of field and cell culture adapted Ranipet isolate of sheep pox virus'. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Snedecor G W and Cochran A W. 1967. *Statistical Methods.* Kolkata (India): Oxford and IBH Publications.

Yathinder P V. 1984. 'Studies on modified sheep pox vaccine-field trials with lamb testes adapted virus'. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Indian Journal of Animal Sciences 73 (10): 1099-1101, October 2003

Clinico-haematological, biochemical, endocrinological and ultrasonographic findings in canine babesiosis

J P VARSHNEY[1], V P VARSHNEY[2] and M HOQUE[3]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 20 April 2002; Accepted: 9 June 2003

ABSTRACT

Clinico-haematological, biochemical, endocrinological and ultrasonographic findings were recorded in 100 clinical cases of canine babesiosis due to *Babesia gibsoni* infection. Cases of babesiosis were scattered throughout the year but 60% were observed from March to June (summer). The disease was in acute form in pups and in chronic form in adults. Pyrexia was inconsistent and haemoglobinuria, icterus and ataxia were of rare occurrence. A wide range of variations were observed in haemoglobin (2 to 11.8 g/dl), haematocrit (6 to 36%), total erythrocyte (0.48 to 4.84 $\times 10^6/\mu l$) and total leukocyte counts (1.4 to 22.05 $\times 10^3 \mu l$). Serum alkaline phosphatase (28 to 400 U/L), ALT (40 to 460 U/L) and AST (50 to 520 U/L) activity values also varied from case to case. The mean values for tri-iodothyronine (0.287±0.005 ng/ml) and thyroxine (13.22±2.22 ng/ml) were markedly low. Sonography revealed both hepato and splenomegaly. Diminazine aceturate @3.5 mg/kg b.wt. intramuscularly on 2 consecutive days or 7.5 mg/kg b. wt. as a single dose was found clinically effective.

Key words: Alanine aminotransferase, Aspartate aminotransferase, Babesiosis, *Babesia gibsoni*, Canine, Endocrinology, Ultrasonography

Canine babesiosis is a tick-borne haemoprotozoan disease of domestic dogs and wild canidae in tropical and subtropical regions of the world. In India, both *B. canis* (Kalra and Singh 1984) and *B. gibsoni* (Sinha and Ghosh 1986, Verma *et al.* 1989, Varshney and Dey 1998) infections are prevalent. But detail systematic studies in *B. gibsoni* infection are lacking. Therefore, the objective of the present study is to present clinico-haematological, biochemical, endocrinological and ultra-sonographic findings associated with infection of *B. gibsoni* in dogs in India.

MATERIALS AND METHODS

Over a period of 5 years (July 1996 to June 2001), 100 dogs with *B. gibsoni* infection were recorded at Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, India. Morphological variants of the parasite were recorded in Giemsa-stained blood smears and the presence of free parasite was also noted. Number of parasitized erythrocytes amongst total erythrocytes in a particular field was assessed. History and detail clinical findings were recorded.

Haematological investigations on venous blood comprising haemoglobin (Hb), haematocrit (Ht), total erythrocyte count (TEC), total leukocyte count (TLC), absolute neutrophil count, absolute lymphocyte count and thrombocyte count were carried out as per standard methods (Jain *et al.* 2000). Serum alkaline phosphatase (SAP), aspartate aminotransferase (AST), and L-alanine aminotransferase (ALT), were estimated colorimetrically using kits. Serum total thyroxine (T_4) and tri-iodothyronine (T_3) were estimated using radio-immuno assay (RIA) kits supplied by Bhabha Atomic Research Centre, Trombay.

Hepatic and splenic ultrasonography was performed with scanner using a 5.0 MHz AAS transducer. After shaving the abdominal area and liberal application of acoustic gel, imaging was performed in dorsal right and lateral intercostal approach using sagittal, transverse and dorsal planes. The distance from skin surface to the mid point of diaphragm was measured with the help of built in caliper to measure liver size. The liver size of healthy dogs of corresponding body weights was calculated using a regression equation (liver size in cm = 4.14±0.19 × body weight in kg) given by Bhadwal *et al.* (1999). The size of the spleen was estimated subjectively comparing the caudal position of the spleen to costal arch. The images were recorded on thermal printing paper using a videocopy processor. Blood pressure was recorded in severely ailing dogs, as per Gaur and Varshney (2002).

Diminazine aceturate was used @3.5 mg/kg b.wt. intramuscularly for 2 consecutive days in 60 cases or @ 7.5 mg p/kg b.wt. as single dose in 37 cases. Dogs (8) were also

Present address: [1]Principal Scientist, Division of Medicine; [2]Principal Scientist, Nuclear Research Laboratory, Division of Physiology and Climatology; [3]Senior Scientist, Division of Surgery.

given doxycycline @ 10 mg/kg b.wt. orally for 14 days in addition owing to concurrent infection of *Ehrlichia canis*. Supportive therapy included blood transfusion (350-500 ml) in 5 dogs having haemoglobin less than 4.0 g/dl. The blood was infused after major crossmatching. Fluid, haemopoietics and hepatostimulants were given as an adjunct therapy.

RESULTS AND DISCUSSION

This study describes clinical, haematological, biochemical, endocrinological and sonographic features of naturally occurring cases of canine babesiosis due to *B. gibsoni* infection in India. During 5 years, from July 1996 to June 2001, *B. gibsoni* infection was diagnosed in 100 clinically ill dogs. Of these 8 dogs had concurrent infection of *E. canis*. The age of dogs ranged from 05 days to 14 years. Dogs 58% were above 24 months and only 14% were up to 6 months of age. The occurrence of *B. gibsoni* in youngs was lower than that of *B. canis* reported by Farwell *et al.* (1982) and Abdullahi *et al.* (1990). However, *B. gibsoni* infection was detected in blood smears of 2 pups, aged 5 days, with acute illness. Possibly, these pups acquired infection through transplacental transmission (Farwell *et al.* 1982, Itoh and Itoh 1990).

Though, the cases of *B. gibsoni* infection were recorded throughout the year, but 60% cases were in summer (March to June) followed by 25 and 15% cases in rainy season and winter respectively. The higher incidence of *B. gibsoni* infection in summer, in the present study, seems to be because of conducive environment for the propogation of tick vectors. Clinical cases of *B. gibsoni* recorded were in 14 breeds of dogs comprising Pomeranian (40), Spitz (03), Lhasa Apso (01), Dachshund (01), Doberman pinscher (20), German shepherd (12), Great Dane (05), Golden retriever (06), Alsatian (4), Boxer (01), Bhutia (2), Dalmation (02), St. Bernard (01) and Labrador (02). Of these ailing dogs 60 were males and 40 were females.

The disease was observed in acute form in pups and chronic in adults. The most common clinical symptoms were inappetance or anorexia, lethargy and recurrent fever, pallor mucus membrane, pyrexia, ticks on body, emesis and gradual emaciation. Ascites was seen in only 10% cases. Subnormal temperature (< 37°C), shock, recumbancy, nervous signs including muscular twitching, ataxia, abdominal pain, epistaxis, jaundice, dark brown urine were seen occasionally. Bilateral hind limb odema with ascites was seen in 3 cases only. From clinical variants it appeared that pyrexia was not a consistent finding and haemoglobinuria, icterus, ataxia occurred rarely in *B. gibsoni* infection agreeing with Chau (1995). Systolic blood pressure was slightly higher (150.50±11.29 mm Hg) in 4 cases and lower (75.86±4.98 mm Hg) in 15 cases as compared to mean values reported for healthy dogs by Gaur and Varshney (2002). Heart rate in dogs having higher SAP was low (120.0±1.35 bpm) and high (186±4.78 bpm) in dogs having low SAP. Probably hypotension caused tachycardia, as a compensatory mechanism.

A wide range was observed in haemoglobin (2.0 to 11.8 g/dl), haematocrit (6.0 to 36.0%), total erythrocytes (0.48 to 4.84 × 10^6 per µl) and total leukocyte (1.4 to 22.05 × 10^3 per µl). Based on haemoglobin values and clinical symptoms cases were classified as acute (severe anaemia) or chronic illness (chronic-anaemia). Young ones (4 cases) had severe anaemia having haemoglobin as 2.0 g/dl with haematocrit value as 6.0% and were in the state of shock. Haemolytic plasma was seen in many cases. Blood films demonstrated anisocytosis, polychromasia, poikilocytosis, nucleated erythrocytes and clumping of erythrocytes suggesting regenerative anaemia. Leukocyte count was variable In 5 cases marked leukopenia and in 10 dogs neutrophilic leukocytosis was evident (Buckner and Ewing 1974). Thrombocytopenia (0.024 to 0.240 millions/µl) was more pronounced in severely ill dogs as also observed by Moore and Williams (1979) in dogs infected with *B. canis*. The minimum (<5%) and maximum haemolysis (>90%) of erythrocytes of ailing dogs were higher (0.75% and 0.35%), respectively, as compared to healthy dogs (0.6 and 0.3%). The present findings agree with those of Makinde and Bobade (1994) in *B. canis* infection. The parasitaemia ranged from 1 to 5% in infected dogs. Itoh *et al.* (1987) reported 0.3 to 7.4% parasitaemia in dogs infected in *B. gibsoni*. Mostly trophozoites were annular, oval, coma or band shaped. A few of *B. gibsoni* were also seen in free state in plasma. In 1 dog, organisms were also found in neutrophils of peripheral blood. Simpson (1974) has reported the presence of *B. canis* in neutrophils of peripheral blood. Multiple parasitization of single erythrocyte with 2 or more parasites was seen in 40 dogs and the rest had 1 parasite.

Results of serum enzymes analysis of 30 dogs with natural infection of *B. gibsoni* are presented in Table 1. There were wide variations as higher values of alkaline phosphatase, AST and ALT. Increase in SAP, ALT and AST values was suggestive of liver involvement. Namikawa *et al.* (1994) found that increases in SAP values was related with clinical severity of the disease in experimental *B. gibsoni* infection. The mean values of tri-iodothyronine (0.287±0.005 ng/ml) and thyroxine (13.22±2.22 ng/ml) were markedly low in diseased dogs as compared to those in healthy dogs. Low levels of thyroid hormones without clinical signs indicated euthyroid condition of secondary nature possibly caused by disturbance of hypothalamic pituitary thyroid axis (Abebe and Eley 1992).

Ultrasonographic examination of liver, of 14 cases revealed a diffuse fine hypoechoic echotexture with prominent vessels and significantly ($P<0.01$) enlarged liver (8.41±0.48 cm) as compared to the calculated normal liver size (6.74±0.34 cm). Hypoechoic changes and increase in liver size were suggestive of inflammation and hepatomegaly (Gosnik *et al.*.1979, Sakuma *et al.* 1987). The spleen was diffusely hyperechoic and enlarged extending up to right paralumbar area and below mid abdomen suggesting inflammation and splenomegaly and

Table 1. Haemato-biochemical indices in dogs having natural infection of *B. gibsoni* (mean ± SE)

Parameters	Values
*Haematological**	
Haemoglobin (g/dl)	4.58±1.23 (2.0 to 11.8)
Haematocrit (%)	15.87±5.66 (6.0 to 36.0)
Total erythrocyte count ($\times 10^6/\mu l$)	1.73±0.139 (0.48 to 4.84)
Total leukocytes count ($\times 10^3/\mu l$)	7.44±4.06 (1.4 to 22.05)
Absolute neutrophil count ($\times 10^3/\mu l$)	4.45±2.62 (0.8 to 15.87)
Absolute lymphocyte count ($\times 10^3/\mu l$)	2.39±0.98 (0.49 to 6.72)
Thrombocyte count ($\times 10^6/\mu l$)	0.1091±0.0055 (0.024 to 0.240)
*Biochemical***	
Serum alkaline phosphatase (SAP) (U/l)	240.53±17.31 28.0-400)
Aspartate aminotransferase (AST) (U/l)	294.33±30.26 (50-520)
Alanine aminotransferase ALT (U/l)	296.3±19.30 (40-460)

Figures in parenthesis indicate range; * n = 40, ** n = 30.

could be ascribed to chronic inflammation (Lijour 1987).

Diminazine aceturate proved effective in 97 cases but failed in clearing *B. gibsoni* infection totally (Farwell *et al.* 1982). Follow up checking of blood smears showed the presence of the organism in 80/97 cases up to 4 to 6 days post treatment. In 8 cases, weakness, incoordination and paresis were observed as post-treatment complications, that disappeared gradually with the supportive treatment within a fortnight. Eight out of 97 dogs, having concurrent infection of *E. canis*, showed erratic response to diminazine therapy alone, but showed uneventful clinical recovery with the addition of doxicycline. Blood transfusion was used only in 5 dogs whose haemoglobin was less than 4.0 g/dl. Ringers lactate, glycerinated haemoglobin with essential minerals (haematinic), tricholine citrate with sorbitol (hepato-tonic) proved good supportives.

It can be inferred from the study that canine babesiosis was associated with hepato and splenomegaly; euthyriodism and inconsistent clinical signs and variations in serum enzyme activity. Diminazine aceturate @ 3.5 mg/kg b.wt. on 2 consecutive days or 7.5 mg/kg b.wt. as a single dose was clinically effective.

REFERENCES

Abdullahi S U, Mohammed A A, Trimnell A R, Sannuri A and Alafiatayo R. 1990. Clinical and haematological findings in 70 naturally occurring cases of canine babesiosis. *Journal of Small Animal Practice* **31**: 145-47.

Abebe G and Eley R M. 1992. Trypanosome induced hypothyroidism in cattle. *British Veterinary Journal* **148**: 63-70.

Bhadwal M S, Mirakhur K K and Sharma S N. 1999. Ultrasonographic imaging of normal canine liver and gall bladder. *Indian Journal of Veterinary Surgery* **20**: 10-14.

Buckner R G and Ewing S A. 1974. Canine babesiosis. *Current Veterinary Therapy V: Small Animal Practice*. pp.984-85. (Ed.) Kirk, R W, W.B. Saunders, Philadelphia.

Chau S R. 1995. Clinicopathological studies on mixed infections with *Babesia gibsoni* and *Ehrlichia canis* in dogs. *Journal of Chinese Society for Veterinary Medicine* **21**: 169-77.

Farwell G E, LeGrand E K and Cobb C C. 1982. Clinical observations on *Babesia gibsoni* and *Babesia canis* infections in dogs. *Journal of American Veterinary Medical Association* **180**: 507-11.

Gaur T and Varshney J P. 2002. Non-invasive measurement of blood pressure and prevalence of blood pressure anomalies in dogs. *Indian Journal of Animal Sciences* **72**: 68-69.

Gosnik B B, Lemon S K, Scheble W and Leopold G R. 1979. Accuracy of ultrasonography in diagnosis of hepatocellular disease. *American Journal of Radiology* **133**: 19-23.

Itoh N, Higuchi S and Ogasawara T. 1987. An outbreak of canine babesiosis in Aomori prefecture. *Journal of Japanese Veterinary Medical Association* **40**: 167-71.

Itoh N and Itoh S. 1990. A case of canine, babesiosis possibly developed by transplacental infection. *Journal of Japanese Veterinary Medical Association* **43**: 275-76.

Jain N C, Feldman B F and Zinkl J G. 2000. *Schalm's Veterinary Haematology*. 5th edn. Lippincott Williams and Wilkins.

Kalra I S and Singh K B. 1984. Observations on naturally occurring *Babesia canis* infection in dogs. *Indian Veterinary Journal* **61**: 798-800.

Lijour L. 1987. L'echotomographic splenique chezles carnivores domestiques. *Pratique Medicale et chisurgicale de l'Animal de compagnie* **22**: 291-302.

Makinde M O and Bobade P A. 1984. Osmotic fragility of erythrocytes in clinically normal dogs and dogs infected with parasites. *Research in Veterinary Science* **57**: 343-48.

Moore D J and Williams M C. 1979. Disseminated intravascular coagulation - a complication of *Babesia canis* infection in the dog. *Journal of South African Veterinary Association* **50**: 265-75.

Namikawa K, Ishibashi T, Sunaga F and Kanno Y. 1994. Diagnosis of severe *Babesia gibsoni* infection in dogs by determination of serum alkaline phosphatase. *Journal of the Japan Veterinary Medical Association* **47**: 415-18.

Sakuma S, Ishiqaki T and Takenchi T. 1987. *Diagnostic Imaging of the Liver, Billiary Tract and Pancreas. Data Analysis and Diagnostic Procedures*. pp. 13-53. Springer Verlag, Berlin.

Simpson C F. 1974. Phagocytosis of *Babesia canis* by neutrophils in peripheral circulation. *American Journal of Veterinary Research* **35**: 701-04.

Sinha B P and Ghosh P. 1986. Treatment of clinical cases of babesiosis. *Indian Journal of Veterinary Medicine* 6: 94-97.

Varshney J P and Dey S. 1998. A clinical study on haemoprotozoan infections in referral canine. *Journal of Remount and Veterinary Corps* **37**: 83-89.

Verma S P, Srivastava P S, Sinha S R P, Sinha B P and Sinha V K. 1989. An outbreak of canine babesiosis in police dog squad of Patna. *Indian Journal of Animal Health* **28**: 47-49.

Indian Journal of Animal Sciences 73 (10): 1102-1104, October 2003

Progesterone and oestradiol profile in postpartum anoestrous cows following crestar and PMSG administration

H C NATH[1], D J DUTTA[2], A DUTTA[3] and R K BISWAS[4]

Assam Agricultural University, Khanapara, Guwahati, Assam 781 022 India

Received: 3 October 2002; Accepted: 2 June 2003

ABSTRACT

Postpartum anoestrous cows (10) were administered with crestar ear implant at the base of the ear followed immediately by 2 ml crestar intramuscular injection for 9 days and folligon (PMSG) @ 300 IU intramuscularly on the day of implant removal. All treated cows that exhibited oestrus were subject to artificial insemination. Mean blood serum progesterone and oestradiol-17ß profile prior to hormonal treatment were 1.87±0.01 ng/ml and 18.33±2.05 pg/ml, respectively, in postpartum anoestrous cows. Thereafter, the progesterone level declined and reached its nadir 0.59±0.03 ng/ml on day of induced oestrus. Animals that conceived following induced oestrus the serum progesterone level reached a peak level of 6.51±0.08 ng/ml on day 12 post onset of induced oestrus and maintained its higher level thereafter. However, the animals that did not conceive the peak level of 6.20±0.04 ng/ml progesterone failed to maintain; instead the level decreased and reached as low as 0.64 ng/ml on day 21 post onset oestrus resulting in subsequent oestrus. The serum oestradiol-17ß level was higher immediately after crestar insertion in the treated cows and thereafter reached a peak level of 92.2 to 97.75 pg/ml on the day of induced oestrus. Serum oestradiol profile recorded a subsequent higher level (30.50±1.82 pg/ml) on day 21 post onset of induced oestrus in animals that failed to conceive, whereas the oestradiol profile reached pretreatment level in animals that conceived.

Key words: Anoestrus, Cow, Crestar, PMSG, Oestrus, Oestradiol-17ß, Progesterone, Postpartum

Anoestrus is the single largest cause of infertility which is caused by various factors. The inhibitory effects of nutritional status, season, suckling and lactation on the reproductive system are mediated by endocrine system and probably via a common final mechanism (Peters and Lamming 1984). Ovarian activity can be induced successfully with progestogen treatment (Peters and Lamming 1986). Gonadotropin with progestogen is frequently used to improve conception rate at the induced oestrus to stimulate follicular growth and ovulation with good oestrus response and conception rate (Mulvehill and Sreeman 1977, Singh *et al.* 1995). The present study was undertaken to assess the pattern of serum progesterone and oestradiol-17ß profile in norgestomet primed and PMSG administered postpartum anoestrous indigenous cows of Assam.

MATERIALS AND METHODS

Indigenous postpartum anoestrous cows (10) with a history of normal calving that had no signs of oestrus 90 days following parturition with smooth ovaries on rectal palpation were included for the present experiment. The cows were maintained under semi-intensive system of rearing with standard managemental practices. Clinico-gynaecological examination was carried out in all cows as per Zemjanis (1970) before and after treatment.

The selected cows were subjected to crestar ear implant containing 3 mg norgestomet at the base of the ear followed immediately by 2 ml crestar intramuscular injection containing 3 mg norgestomet and 5 mg oestradiol valerate. The day of implant insertion was considered as day of treatment. The implant was removed on day 9 of the treatment and folligon (pregnant mare serum gonadotropin) @ 300 IU was administered intramuscularly to each treated animal.

The animals were observed for occurrence of oestrus following implant removal with the help of a vasectomised bull and inseminated with good quality semen during 8-10 hr and 15-17 hr after the onset of oestrus. The conception was confirmed by rectal palpation on day 45 of insemination and serum progesterone profile.

Blood serum was collected for the estimation of serum progesterone and oestradiol-17ß from all the animals just before implant insertion, day 5 of implant insertion, on the day of implant removal, at oestrus and then at 3 days interval

Present address: [1]Research Associate, [2]Associate Professor, [3]Assistant Professor, Department of Animal Physiology, [4]Associate Professor, Department of Gynaecology, Obstetrics and AI, College of Veterinary Science.

until 30 days post oestrus. Serum progesterone and oestradiol-17ß concentrations were estimated by radioimmuno assay (RIA) using Coat-A–Count progesterone RIA kit in 1-125 gamma counter following standard procedure.

RESULTS AND DISCUSSION

The pattern of serum progesterone and oestradiol-17ß profile during different days prior to and after crestar and PMSG administration is shown in Fig. 1. The mean serum progesterone concentration prior to treatment in animals was 1.87±0.10 ng/ml. Higher average serum progesterone concentration of more than 1 ng/ml was also recorded by Lamond *et al.* (1971) in anoestrous cows while Smith *et al.* (1987) and Rao *et al.* (1989) recorded lower value of 0.3 to 0.4 and 0.60 to 0.78 ng/ml respectively. The difference in progesterone level could be attributed to presence of varying amount of luteal tissue in animals. The higher serum progesterone concentration in the present study might be attributed to luteinization of an unovulated follicle resulting in transient progesterone rise (Peters and Lamming 1984).

Following hormonal treatment, the mean progesterone level slightly declined from pretreatment level to 1.11±0.02 ng/ ml on day 5 in cows that latter conceived and 1.06±0.03 ng/ml that did not conceive. On the day of progesterone withdrawal i.e., on day 9 of crestar implant, the mean level was 0.72±0.02 ng/ml and 0.75±0.02 ng/ml in cows that conceived and not conceived respectively. The mean concentration of serum progesterone reached its nadir (0.61±0.02 and 0.56±0.04 ng/ml) on the day of induced oestrus following treatment in conceived and non-conceived animals respectively. Stevens *et al.* (1992) reported that circulating plasma progesterone concentration was greater than 1.0 ng/ml for every postpartum cows prior to hormonal treatment and declined to values less than 1.0 ng/ml by 24 hr after $PGF_2\alpha$ treatment. Dobson *et al.* (1973) also reported

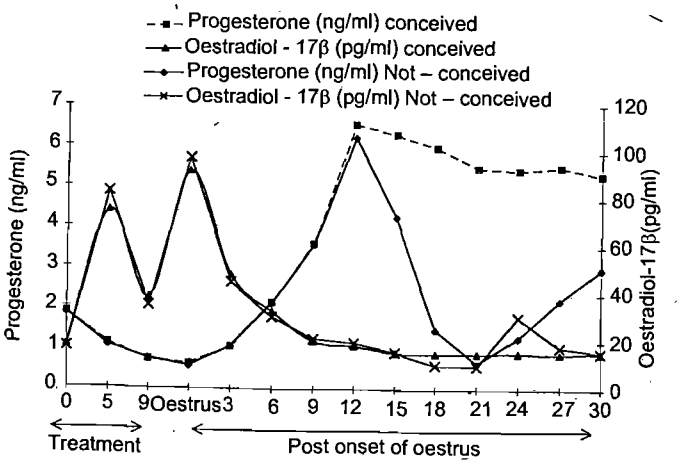


Fig 1. Serum progesterone and oestradiol-17ß concentration in postpartum anoestrous cows during different days prior to and after hormonal treatment.

that serum progesterone concentration declined significantly following melengestrol acetate treatment in Friesian cows. The lower concentration of serum progesterone concentration on the day of oestrus was in agreement with Dobson *et al.* (1973), Agarwal *et al.* (1977) and Andurkar *et al.* (1997).

Following oestrus the mean serum progesterone concentration gradually increased and reached a peak level of 6.51±0.08 and 6.20±0.04 ng/ml on the day 12 post onset of oestrus in conceived and non-conceived animals, respectively, which reflected the normal development of corpus luteum following ovulatory oestrus. The results are in agreement with that of Booth *et al.* (1975), Kudlac (1981) and Rao *et al.* (1989) who made similar observation. In this study although 9 cows were bred at induced oestrus only 5 cows conceived and they maintained elevated serum progesterone concentration till 30 day of observation (Fig.1) but the remaining 4 animals did not maintain the elevated level of progesterone (Fig. 1) during luteal phase which rather gradually declined to 0.64±0.05 ng/ml on day 21 post onset of oestrus. This indicated failure of conception and resumption of cyclicity with onset of oestrus in the subsequent cycle. The subsequent rise in serum progesterone level on days 24, 27 and 30 post onset of induced oestrus along with the observed behavioural oestrus around day 21 post onset of oestrus in the 4 treated cows corroborated the resumption of ovarian cyclicity.

The mean serum oestradiol-17ß concentration prior to treatment in experimental animals was 18.33±2.05 pg/ml. The level was similar with the findings of Rao *et al.* (1989) in postpartum anoestrous cows. Madan *et al.* (1983) reported plasma estradiol level ranging between 10.00 to 37.50 pg/ml in true anoestrous animal. However, lower serum concentration of oestradiol was also reported in postpartum anoestrous cows (Mondhe *et al.* 1989). This could be due to difference in age, body weight, nutrition and ovarian status of individual cows in different studies. Following hormonal treatment mean serum oestradiol-17ß concentration rose sharply to 75.4±2.01 and 83.5±3.40 pg/ml in treated cows that later conceived and not-conceived, respectively, which might be due to presence of oestradiol valerate incorporated in the crestar injection. Rise in serum oestradiol level immediately following exogenous application of oestradiol-17ß and GnRH was also observed by Rao *et al.* (1989) in postpartum anoestrous cows. Thereafter it declined on day 9 of treatment to a level of 38.8±2.73 and 35.25±3.75 pg/ml, respectively, in conceived and non-conceived animals.

On the day of induced oestrus following crestar administration the mean serum oestradiol-17ß concentration reached a peak level of 92.2±4.07 and 97.75±5.58 pg/ml in cows that conceived and not-conceived respectively. Wettemann *et al.* (1982) recorded as high as 25-30 pg/ml plasma oestradiol concentration at oestrus following PMSG treatment as compared to 12-14 pg/ml in GnRH-treated postpartum anoestrous cows at induced oestrus. Higher serum

oestradiol concentration on the day of induced oestrus in the present study might be due to administration of progesterone-oestrogen combination along with PMSG which might have influenced steroidogenic and ovarian follicular activity in the treated animals.

Our results revealed that crestar implant for 9 days with PMSG on the day of crestar withdrawal was optimum for bringing the postpartum anoestrous cows into oestrus and ovulation. The progesterone and oestradiol profile between treated cows that later conceived or not did not reveal wide variation in hormonal pattern, however, the profile in induced oestrus differed from that of natural oestrus. Animals that failed to conceive following treatment resumed natural cyclic activity.

ACKNOWLEDGEMENTS

We thank Dr K K Baruah, Associate Professor cum RSO, NRL, Department of Animal Physiology, for estimation of hormones. We also thank Dr B C Sarmah, Professor and Head, Department of Animal Physiology, College of Veterinary Science, for providing facilities for this study.

REFERENCES

Agarwal S P, Rahman S A, Laumas K, Agarwal V K and Ahmed A. 1977. Studies on steroid hormones: progesterone concentration in the blood serum of zebu cows during oestrous cycle. *Indian Journal of Animal Sciences* **47** (2): 715-19.

Andurkar S B, Kadu M S, Chinchkar S R and Sadekar R D. 1997. Serum progesterone profile in buffaloes treated with CIDR-Device and combinations. *Indian Journal of Animal Reproduction* **2**: 104-07.

Booth W D, Newcomb R, Strange H, Rowson L E A and Sacher H B. 1975. Plasma oestrogen and progesterone in relation to superovulation and egg recovery in the cow. *Veterinary Records* **97**: 366-69.

Dobson H, Boyd L J and Exky D. 1973. Circulating oestrogns and progesterone in the bovine after synchronization with melengestrol acetate. *Journal of Reproduction and Fertility* **33**(2): 231-37.

Kudlac E. 1981. Inducting fertile oestrus in anoestrous cows using a synthetic analogue of $PGF_2\alpha$ oestrogen injection (SPOFA). *Biologizace a chemizace Zivocisne Vyroby Veterinaria* **27**(91): 51-60.

Lamond D R, Dickey J F, Henricks D M, Hili J R and Leland T M. 1971. Effect of progesterone on the bovine ovary. *Journal of Animal Science* **33**: 77.

Madan M L, Naqvi S M K, Trien C V, Suri A K and Prakash B S. 1983. Plasma oestradiol-17ß, progesterone and cortisol among anoestrus rural animal. *Indian Journal of Animal Reproduction* **3**(1): 60.

Mondhe D M, Deopurkar V L, Hukeri V B, Mantri A M, Hingana V R and Kaikini A S. 1989. Serum FSH and oestradiol-17ß concentrations in postpartum anoestrous crossbred cattle. *Indian Journal of Animal Reproduction* **10**(1) L 6-9.

Mulvehill P and Sreeman J M. 1977. Improvement of fertility in postpartum beef cows by treatment with PMSG and progestogen. *Journal of Reproduction and Fertility* **50**: 323-25.

Peters A R and Lamming G E 1984. Reproductive activity of the cow in the postpartum period. 2. Endocrine patterns and induction of ovulation. *British Veterinary Journal* **140**: 269-79.

Peters A R and Lamming G E. 1986. Regulation of ovarian function in the postpartum cow: An endocrine model. *Veterinary Records* **118**: 236-39.

Rao N A V, Palmor W M and Sheikeldin M A. 1989. Gonadotropin releasing hormone induced oestrus. Hormonal and vaginal resistance changes and their temporal relationship in anoestrous cows pretreated with and without beta oestradiol. *Indian Veterinary Journal* **66**: 1137-40.

Singh Umed, Khar S K, Khurana N I, Indrajeet and Nanda T. 1995. Fertility of anoestrus zebu cattle to progesterone therapy. *Symposium on Research and Management of Surgical and Reproductive Disorders in Veterinary Practices*, held at Hisar. Pp. 27-34.

Smith V G, Chenault J R, Meallister J F and Auderdale J W. 1987. Response of postpartum beef cows to exogenous progesterone and gonadotropin releasing hormone. *Journal of Animal Science* **67**(2): 540-51.

Stevens R D, Seguen B E and Moment H W. 1992. Simultaneous injection of PGF_2 and GnRH in to diestrous dairy cows delays return to oestrus. *Theriogenology* **39**: 373-80.

Wettemann R P, Beek T W, Turman E J and Hintz R L. 1982. Endocrine response of postpartum anoestrous beef cows to GnRH or PMSG. *Theriogenology* **18**(5): 599-13.

Zemjanis S R. 1970. *Diagnostic and Therapeutic Technique in Animal Reproduction*. 2nd edn. The Williams Wilkins Co., Baltimore.

Indian Journal of Animal Sciences **73** (10): 1105-1110, October 2003

Effect of camel milk on glycemic control, lipid profile and diabetes quality of life in type 1 diabetes: A randomised prospective controlled cross over study

R P AGRAWAL[1], S C SWAMI[2], R BENIWAL[3], D K KOCHAR[4], M S SAHANI[5], F C TUTEJA[6] and S K GHORUI[7]

SP Madical College, Bikaner, Rajasthan 334 003 India

Received: 28 August 2002; Accepted: 30 May 2003

ABSTRACT

The effects of camel milk on glycemic control, lipid profile and diabetes quality of life in type 1 diabetic patients, were evaluated randomly selected type 1 diabetic patients (24) were divided into 2 groups. Group 1 (N=12) received usual care i.e. standardized diet, standardized exercise regimen and insulin for 3 months. Then camel milk (500 ml) was added for next 3 months. Patients of group 2 (N=12) received camel milk (500 ml) and usual care for first 3 months and only usual care in next 3 months. Frequent blood glucose monitoring was done to keep euglycemic status by titrating the dose of insulin. Analysis of HbA_1c, lipid profile, insulin and C-peptide was done in the beginning, at the end of third and sixth month. In group I patients the requirement of insulin was 40.83±7.12 at the beginning, 41.67±5.49 after 3 months and it reduced to 26.83±8.44, ($P<0.05$) after camel milk supplementation. In group 2 dose of insulin increased from 30.00±13.01 to 40.57±15.20 when camel milk was withdrawn. Improvement was observed in glycemic control; in group 1 HbA_1c reduced from 9.48±2.17 to 8.19±1.84 and in group 2 HbA_1c decreased from 9.59±1.62 to 8.02±1.17. Statistically significant improvement was seen in D.Q.L. score. It is concluded that moderate intake of camel milk will reduce the insulin requirement with better glycemic control and diabetes quality of life without affecting lipid profile.

Key words: Alternative therapy, Camel milk, Diabetes

Type 1 diabetes mellitus is an organ specific auto immune disease, characterized by chronic hyperglycemia and disturbances of carbohydrates, fat and protein metabolism associated with insulin deficiency. Cow milk feeding induces primary immunization to insulin in infants who are at genetic risk for type 1 diabetes (Vaarala *et al.* 1999). The incidence of diabetes mellitus appears to be increasing (Onkamo *et al.* 1999) all over the world. Prevention and early treatment is important because diabetes interrupts normal developments in children and carries the threat of severe complications in more active period of life (Beg *et al.* 1986). Insulin replacement is the mainstay of treatment but at present, entire physiological insulin replacement can not be achieved in clinical practice and metabolic disturbances can not be normalized. Insulin therapy is still the best treatment but in our country needle phobia and cost of the treatment forces these patients to adopt alternative treatments. In this connection, there are many folklore stories describing the use of camel milk in type-1 diabetes mellitus. The camels in India are mostly used for drought purpose. However, good genetic potential to produce milk exists (Khanna and Rai 1993). There is also an account in memories of emperor Jahangir (1579 - 1627 AD) about usefulness and acceptability of camel milk (Rogers 1989). One of the camel milk proteins have many characteristics similar to insulin (Dahlquist 1999), and it does not form coagulum in acidic environment (Wangoh 1993). This lack of coagulum formation allows the camel milk to pass rapidly through the stomach together with the specific insulin like protein/insulin and remains available for absorption in intestine. Radioimmunoassay of camel milk has revealed high concentration of insulin i.e. 52 units/litre (Singh 2001; personal communication). The concentration of insulin in human milk is also significantly higher (60.23 ± 41.05 micro µ/ml), whereas, it is very low in cow milk (16.32 ± 5.98 micro µ/ml)(DCCT 1996) but probably due to coagulation of these proteins in the stomach it may not be available for absorption in the intestine.

The effects of camel milk on glycemic control, lipid profile and diabetes quality of life in type 1 diabetic patients were evaluated.

MATERIALS AND METHODS

Type 1 diabetic patients (24) were randomly recruited from

Present address: [1]Assistant Professor, [2]Senior Registrar, [4]Professor, Department of Medicine; [3]Senior Registrar, Department of PSM.

[5]Director, [6]Senior Scientist, [7]Scientist National Research Centre on Camel, Jorbeer, Bikaner.

the outpatient diabetic clinic in PBM hospital, Bikaner, India. Ethical committee of S P Medical College, Bikaner, approved the protocol and all subjects gave written consent before participation in the study. Patients with any acute metabolic complications like hypoglycemia, ketoacidosis, cardiovascular event, renal or acute infections were not included in the study. Eligible patients entered a run–in period of 1 month in which they were oriented to achieve the best possible glycemic control through standardized diet, standardized exercise regimen and insulin administration. During this period frequent monitoring of blood sugar was done to maintain euglycemia. At the end of run-in period, a base line evaluation was performed. These patients were again randomly divided into 2 groups. Group 1 patients (N=12) received usual care i.e. diet, exercise and insulin for 3 months, then camel milk 500 ml was added to usual care for next 3 months. The group 2 patients (N=12) received 500 ml of camel milk in addition to usual care for first 3 months and then camel milk was withdrawn in the last 3 months (Fig. 1).

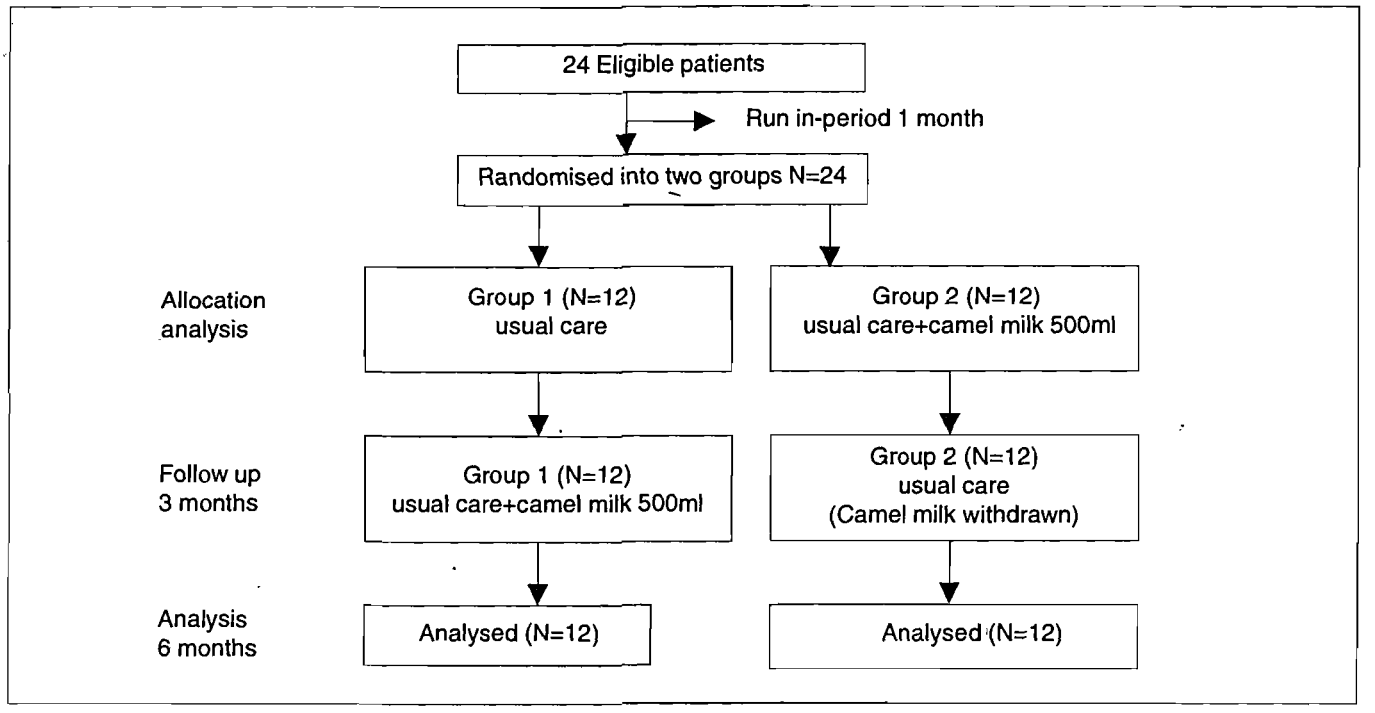


Fig. 1. Study design.

It was a randomized, open case control, crossover clinical trial design. Blood sugar was measured using the glucose oxidase method twice in a week before breakfast and dinner. Plasma insulin, C-peptide, anti insulin antibodies, HbA$_1$c and lipid profile were estimated at the start of the study, at 3 months and at the end of study. Plasma insulin and C-peptide were estimated by fully automated chemi-illuminescence (CLIA) test. Anti-insulin antibodies were estimated by radioimmuno assay. HbA$_1$c was measured by high performance liquid chromatography (HPLC). Plasma total cholesterol, triglycerides, VLDL, HDL, LDL were estimated by fully automated biochemistry analyzer. Urine microalbumin was tested by micral test. Body mass index, waist hip ratio, and diabetes quality of life score were also measured every week (Shehadeh *et al.* 2002, Surwit 1992).

Baseline characteristics of groups 1 and 2 were analyzed using the Mann-Whitney U test. Each group was followed over time and differences arising in variables analyzed using Kruskal Wallis (Beth and Trapp 1993) ANOVA and post-HOC comparisons made using Tukey's *HSD* (Beth and Trapp 1993)(p value <0.05 was considered statistically significant). Results were expressed as mean ± CI unless otherwise stated. SPSS 7.2 was used for analysis.

RESULTS

The groups 1 and 2 were similar in age (18.75 ± 1.91 vs 20.17 ± 7.52), sex (10M, 2F in both groups), body mass index (18.44 ± 3.59 vs 18.83 ± 4.40), mean blood sugar (7.05 ± 0.43 vs 7.18 ± 0.49), plasma insulin (130.32 ± 66.78 vs 123.9 ± 47.76), c-peptide (0.63 ± 0.11 vs 0.61 ± 0.07) plasma lipids along with different clinical, demographical and biochemical variables (Table 3).

Characteristics of camel milk

Milk composition (Tables 1, 2) as revealed by experiments was carried out at the National Research Center on Camel, Bikaner, India (*Annual Report* 1989-90)(Sahni 1989, Willium 1999).

Effect of camel milk on glycemic control: In group 1 there

Table 1. Composition of camel and cow milk

Component	Camel milk	Cow milk
Water	86-91%	88%
Lactose	4.2-5.0 g%	5.0 g%
Fat	2.49-3.1 g%	3.7 g%
Protein	3.78-3.9 g%	3.3 g%
Casein: albumin ratio	2.85	3.1
Sodium	38-68 mg/100ml	35-60 mg/100ml
Potassium	50-90 mg/100ml	135-155 mg/100ml
Magnesium	11.88-13.59 mg/100ml	10-15 mg/100ml
Phosphorus	41.72-47.21 mg/100ml	75-110 mg/100ml
Calcium	94.1-97.43 mg/100ml	100-140 mg/100ml
Iron	0.32-0.36 mg/100ml	0.30-0.65 mg/100ml
Zinc	1.2-6.3 mg/100ml	1.0-3.0 mg/100ml
Manganese	0.02-2.0 mg/100ml	0.01-0.03 mg/100ml
Copper	0.09-0.5 mg/100ml	0.03-0.17 mg/100ml
Vitamin A	0.03 mg%	0.34 mg%
Vitamin C	4-5 mg/100g	1.6 mg/100g
Vitamin E	0.27 mg%	0.1 mg%
Niacin	0.46 mg%	0.08 mg%
Vitamin-B_1	0.03 mg%	0.42 mg%
Vitamin-B_2	0.04 mg%	1.57 mg%

Immunoglobulins: IgM, IgG, IgA and even IgD were detected in camel milk.

was improvement in mean blood glucose (7.11 ± 0.32 to 5.96 ± 0.31, P<0.05), HbA_1c (9.48 ± 2.17 to 8.98 ± 1.84) after addition of camel milk in the treatment regimen. In group 2 there was also an improvement in mean blood glucose (7.18 ± 0.49 to 6.39 ± 0.19) and HbA_1c (9.59 ± 1.62 to 8.02 ± 1.17) but this glycemic control deteriorated slightly when camel milk was withdrawn resulting in mean blood glucose (6.39 ± 0.19 to 6.78 ± 0.18), HbA_1c (8.02 ± 1.17 to 8.78 ± 1.20) (Tables 4,5).

Table 2. Amino acid composition of camel milk and cow milk (Yagil 1985)

Amino acid (g/100 g protein)	Camel milk	Cow milk
Alanine	3.1-3.4	3.5-4.8
Arginine	3.2-4.6	2.9-4.2
Aspartic acid	6.2-7.7	6.2-7.8
Glutamic acid	15.4-23.5	15.8-23.2
Glycine	0.6-6.6	0.8-2.1
Histidine	2.5	3.0
Leucine	18-21	8.1-17.0
Lysine	7.6	8.1
Methionine	3.5	3.2
Phenyl alanine	5.7	5.4
Proline	13.3	10.1-11.8
Serine	5.9	6.6
Threonine	6.3	4.3
Tyrosine	5.8	5.8
Valine	7.4	7.5

Effect of camel milk on insulin dose

Insulin requirement initially and after camel milk are shown in Tables 4,5. In group 1 the dose of insulin reduced from 41.67 ± 5.49 to 26.33 ± 8.44, (P<0.05) when camel milk was added. Similar benefits were observed in group 2 when patients were taking camel milk (41.67 ± 15.65 to 30.00 ± 13.01) but when camel milk was withdrawn there was more requirement of insulin (30.00 ± 13.01 to 40.57 ± 15.20)(Tables 4,5).

Effect of camel milk on diabetes quality of life score

There was statistically significant improvement in diabetes quality of life score when camel milk was added along with usual care. In group 1 patients there was improvement in satisfaction score (26.25 ± 1.96 to 22.25 ± 4.56, P<0.05), impact score (32.58 ± 2.64 to 26.5 ± 3.73, P<0.05) and worry score (14 ± 1.04 to 11.33 ± 1.83, P<0.05). Similar positive response in diabetes quality of life score was seen in group 2 when patients were on camel milk but when it was withdrawn there was deterioration in satisfaction score (27.58 ± 2.19 to 30.00 ± 2.52, P<0.05) without affecting the impact score and worry score (Tables 4,5).

Effect of camel milk on lipid profile, plasma insulin and c-peptide

Fasting plasma insulin and c-peptide levels did not reveal any significant change in either group and similar observation was made with levels of lipid profile (Tables 4,5).

DISCUSSION

The present study was performed to observe the role of camel milk in achieving glycemic control in type 1 diabetes. We observed slight improvement in mean BMI in group 1 (18.21 ± 3.26 to 18.65 ± 3.48) and in group 2 (18.83 ± 4.40 to 20.21 ± 3.99) during camel milk supplementation although it was not significant statistically. The positive effects in weight gain may be because of good nutritional value of camel milk. No other studies are available for comparison. We also observed significant reduction in insulin doses to obtain glycemic control. Requirement of mean doses of insulin/day before treatment in patients of group 1 was 41.67±15.49. It came down very fast initially and then gradually to a mean level of 26.33±8.44 (P<0.05). Similarly in group 2 the mean doses of insulin/day requirement was 41.67±15.65 and it came down after camel milk supplemented to 30.00±13.01 and when milk was withdrawn from the treatment, insulin requirement increased again to 40.57 ± 15.20. The requirement of insulin doses reduced in both the groups when camel milk was added and insulin requirement increases when camel milk was withdrawn in both the groups. It may be because of higher insulin/insulin like protein in camel milk. Camel milk has about 52 units/litre insulin (Singh 2001). Oral insulin has been known since many years but the critical drawback is its coagulum formation in acidic media in stomach, which

Table 3. Base line characteristics

Variables	Group1				Group 2				Mann Whitney U	
	Mean	-95% CI	+95% CI	SD	Mean	-95% CI	+95% CI	SD	Z adj.	p-level
Age	18.75	17.53	19.97	1.91	20.17	15.39	24.94	7.52	-1.13	0.258
BMI	18.44	16.15	20.72	3.59	18.83	16.03	21.62	4.40	-0.35	0.729
HbA c	8.57	7.18	9.96	2.19	9.59	8.56	10.62	1.62	-1.47	0.141
Dose of insulin	40.83	36.31	45.36	7.12	41.67	31.73	51.61	15.65	-0.03	0.977
Mean blood glucose (mmol/l)	7.05	6.77	7.32	0.43	7.18	6.87	7.49	0.49	-0.81	0.417
Micro albuminurea	23	13.81	32.19	14.47	27.80	14.36	41.24	21.15	-0.29	0.773
T.cholesterol (mmol/l)	4.26	4.10	4.42	0.25	4.22	3.77	4.66	0.70	0.00	1.000
HDL (mmol/l)	1.37	1.31	1.43	0.10	1.50	1.37	1.63	0.20	-1.94	0.052
LDL (mmol/l)	2.57	2.27	2.87	0.47	2.25	1.79	2.71	0.72	-1.10	0.273
VLDL (mmol/l)	0.37	0.31	0.43	0.10	0.36	0.27	0.45	0.14	-0.43	0.664
TG (mmol/l)	1.82	1.52	2.13	0.48	1 73	1.31	2.15	0.66	-0.64	0.524
Satisfaction score	28	26.49	29.51	2.37	26.17	24.71	27.62	2.29	-1.69	0.092
Impact	34.33	32.35	36.31	3.11	32.50	30.84	34.16	2.61	-1.29	0.199
Worry	15.5	14.76	16.24	1.17	14.67	13.84	15.49	1.30	-1.51	0.131
C-peptide (nmol/l)	0.63	0.56	0.70	0.11	0.61	0.57	0.66	0.07	-0.38	0.707
Plasma insulin (pmol/l)	130.32	87.9	172.74	66.78	123.9	93.6	154.2	47.76	-0.35	0.729

neutralizes its potency. One property of camel milk is that it does not form the coagulum in the stomach or in the acidic media, it prevents degradation of insulin in the stomach. Beg *et al.* (1986) found that amino acid sequence of some of the camel milk protein is rich in half cystine, which has superficial similarity with insulin family of peptides. There was no significant change in lipid profile i.e. cholesterol, HDL, LDL, VLDL and triglycerides (P< Ns) in both the groups and it may be because of lower fat contents of camel milk (2.49-3.1g % vs cow milk 3.79g %).

It was also observed that after adding camel milk to the usual regimen there was improvement in microalbuminuria. It may be due to good glycemic control or it may be due to direct effect of camel milk. The mechanism behind this effect is still unknown. There was also marked improvement in diabetes quality of life score after 3 months of camel milk treatment. It may be because of good glycemic control or anabolic effect of camel milk. El Agamy (1992) found good amount of lysozyme, lactoferrin, lactoperoxidase, immunoglobulin G and secretory immunoglobulin A in camel milk.

This study was only to see the beneficial effect of camel

Table 4. Metabolic and hormonal characteristics of the group 1 at 0,3 and 6 months

Variables	0 Months		3 Months		6 Months		Kruskal-Wallis ANOVA		Tukey's HSD p value		
	Mean	SD	Mean	SD	Mean	SD	H(2,36)	p value	0-3	3-6	0-6
Age	18.75	1.91	18.75	1.91	18.75	1.19	0	n.s.	n.s.	n.s.	n.s.
BMI	18.44	3.59	18.21	3.26	18.65	3.48	0.169	n.s.	n.s.	n.s.	n.s.
HbA ic	8.57	2.19	9.48	2.17	8.98	1.84	1.14	n.s.	n.s.	n.s.	n.s.
Dose of insulin	40.83	7.12	41.67	5.49	26.33	8.44	15.99	<<0.05	n.s.	<0.05	<0.05
Mean plasma glucose	7.04	0.43	7.11	0.32	5.96	0.31	23.15	<<0.05	n.s.	<0.05	<0.05
Microalbuminurea	23	14.47	121.38	8.22	18.52	6.69	0.64	n.s.	n.s.	n.s.	n.s.
T.cholesterol	4.26	0.25	4.31	0.19	4.28	0.18	0.56	n.s.	n.s.	n.s.	n.s.
HDL	1.37	0.10	1.42	0.10	1.44	0.10	2.98	n.s.	n.s.	n.s.	n.s.
LDL	2.57	0.47	2.59	0.17	2.56	0.15	2.48	n.s.	n.s.	n.s.	n.s.
VLDL	0.37	0.10	0.38	0.09	0.36	0.09	1.33	n.s.	n.s.	n.s.	n.s.
TG	1.82	0.48	1.86	0.44	1.80	0.44	0.47	n.s.	n.s.	n.s.	n.s.
Satisfaction	28	2.37	26.25	1.96	22.25	4.56	12.23	<<0.05	n.s.	<0.05	<0.05
Impact	34.33	3.11	32.58	2.64	26.5	3.73	19.62	<<0.05	n.s.	<0.05	<0.05
Worry	15.5	1.17	14	1.04	11.33	1.83	20.51	<<0.05	<0.05	<0.05	<0.05
C-peptide	0.63	0.11	0.62	0.13	0.62	0.13	0.29	n.s.	n.s.	n.s.	n.s.
Plasma insulin	130.32	66.78	130.26	63.24	127.14	61.92	0.15	n.s.	n.s.	n.s.	n.s.

Values = Mean ± S.D., *P=<0.05.

Table 5. Metabolic and hormonal characteristics of the group 2 at 0, 3 and 6 months

							Kruskal Wallis ANOVA		Tukey's HSD p value		
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	H(2,36)	P value	0-3	3-6	0-6
Age	20.17	7.52	20.17	7.52	20.17	7.52	0	n.s.	n.s.	n.s.	n.s.
BMI	18.83	4.40	20.21	3.99	19.54	3.91	0.79	n.s.	n.s.	n.s.	n.s.
HbA1c	9.59	1.62	8.02	1.17	8.78	1.20	6.95	<<0.05	<<0.05	n.s.	n.s.
Dose of insulin	41.67	15.65	30.00	13.01	40.57	15.20	4.07	n.s.	n.s.	n.s.	n.s.
Mean plasma glucose	7.18	0.49	6.39	0.19	6.78	0.18	18.43	<<0.05	<<0.05	<0.05	<0.05
Microalbuminurea	27.80	21.15	24.45	14.15	26.78	14.14	0.55	n.s.	n.s.	n.s.	n.s.
T. cholesterol	4.22	0.70	4.18	0.64	4.23	0.64	0.21	n.s.	n.s.	n.s.	n.s.
HDL	1.50	0.20	1.45	0.16	1.41	0.16	2.51	n.s.	n.s.	n.s.	n.s.
LDL	2.25	0.72	2.46	0.48	2.50	0.47	0.85	n.s.	n.s.	n.s.	n.s.
VLDL	0.36	0.14	0.34	0.09	0.35	0.08	0.52	n.s.	n.s.	n.s.	n.s.
TG	1.73	0.66	1.78	0.57	1.82	0.38	0.62	n.s.	n.s.	n.s.	n.s.
Satisfaction	26.17	2.29	27.58	2.19	30.00	2.52	13.2	<<0.05	<<0.05	n.s.	<0.05
Impact	32.50	2.61	32.67	2.39	35.08	2.54	7.19	<<0.05	<<0.05	n.s.	n.s.
Worry	14.67	1.30	14.83	1.27	16.92	1.08	15.48	<<0.05	<<0.05	0.05	n.s.
C peptide	0.61	0 07	0.58	0.07	0.58	0.07	0.87	n.s.	n.s.	n.s.	n.s.
Plasma insulin	123.9	47.76	132.12	37.14	132.48	36.84	0.09	n.s.	n.s.	n.s.	n.s.

Values = Mean ± S.D., *P=<0.05.

design and there is a possibility that the effect of one diet carried over into the next diet, thus influencing the results. However a significant carry over effect is not remarkable in our study.

In conclusion the data of this study shows a significant hypoglycemic effect of camel milk when given as an adjunctive therapy. The action is presumed to be due to presence of insulin/insulin like protein in it. Its therapeutic efficacy may be due to lack of coagulum formation of camel milk in acidic media. There is no doubt that the discovery and development of oral insulin for therapeutic use is a himalayan task. It has been observed that oral administration of insulin initiated at clinical onset of type 1 diabetes did not prevent the deterioration of beta cell function. Chaillous *et al.* (2000) and Pozzilli *et al.* (2000) in IMDIAB VII study indicated that addition of 5mg of oral insulin does not modify the course of the disease in the first year after diagnosis and probably does not statistically effect the humoral immune response against insulin. It is important to note that a certain level of scientific testing on camel milk has already been attempted and documented, particularly, insulin levels in camel milk and this scientific wisdom can be a remarkable achievement for diabetic patients.

ACKNOWLEDGEMENT

We thank Dr Raghvendra Singh, Senior Scientist, National Research Center on Camel, Bikaner, for chemical analysis of camel milk.

REFERENCES

Beg O U, Von Bahr, Lindrom H, Zaidid Z H and Jornvall H.1986. A camel milk protein rich in half cystine. Primary structure assessment of variations, internal repeat patterns and relationship with neurophysin and other active polypeptides. *European Journal of Biochemistry* **15**(1): 195-201.

Beg O U, Von Bahr, Lindrom H, Zaidid Z H and Jornvall H. 1986. Characteristic of camel milk protein rich in proline identifies a new beta casein fragment. *Regulatory Peptides* **15**(1): 55-61.

Beth Dawson Saunders and Trapp Robert G. 1993. *Basic and Clinical Statistics II pp.* 133-34.

Beth Dawson Saunders and Robert G Trapp. 1993. *Basic and Clinical Statistics, II pp.* 139.

Chaillous L, Lefevre H, Thivolet C, Boitard C, Lahlou N, Atlan-Gepner C, Bouhanick B, Mogenet A, Nicolino M, Carel JC, Lecomte P, Marechaud R, Bougneres P, Charbonnel B and Sai P. 2000. Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. Diabetes insulin oral group. *Lancet* **356**(9229): 545-49.

Dahlquist G G. 1999. Primary and secondary prevention strategies of pre-type 1 diabetes: potentials and pitfall. *Diabetes Care* 22 (Suppl. 2): B4-B6.

El Agamy E I, Ruppanner R and Ismail A. 1992. Antibacterial and antiviral activity of camel milk protective proteins. *Journal of Dairy Research* **59**(2): 169-175.

Khanna N D and Rai A K. 1993. Milk production potential of Indian camel. FAO, *Asian Livestock* **18**: 19-21.

Onkamo P, Vaananen S, Karvonen M and Tuomilehto J. 1999. World wide increase in incidence of type 1 diabetes: analysis of the data on published incidence trends. *Diabetologia* **42**: 1395-1403.

Pozzilli P, Pitocco D and Visalli N. 2000. No effect of oral insulin on residual beta-cell function in resent-onset Type 1 diabetes (the IMDIAB VII). *Diabetologia* **43**: 1000-04.

Rogers A. 1989. *Memories of Jahangir.* Allantic Publishers and Distributor, New Delhi, 315.

Sahni M S, Mal G, Kumar R, Saina D S and Jain B K 1989. *Annual*

Report on Camel Milk and Its Role in Tuberculosis. NRCC, Bikaner. pp. 7-8.

Shehadeh N, Gelertner L, Blazer S, Perlman R, Solovachik L and Etzioni A. 2002. Importance of insulin content in infant diet: suggestion for a new infant formula. *Acta Paediatr* **90**(1): 93-5.

Surwit R S, Schneider M S and Feinglos M N. 1992. Stress and diabetes mellitus. *Diabetes Care* **15**: 1413-22.

The Diabetes Control and Complications Trial Research Group. 1996. Influence of intensive diabetes treatment on quality of life outcomes in the diabetes control and complications trial. *Diabetes Care* **19**(3): 195-202.

Vaarala O, Knip M, Paronen J, Hamalainen A M, Muona P, Vaatainen M, Ilonen J, Simell O and Akerblom H K. 1999. Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type I diabetes. *Diabetes* **48**(7): 1389-94.

Wangoh J. 1993. What steps towards camel milk technology? *International Journal of Animal Science* **8**: 9-11.

Willium F Ganong. 1999. *Review of Medical Physiology.* 19th edn, pp. 431.

Yagil R. 1985. *The Desert Camel: Comparative Physiology*. Vol. 5. Co., Animal Nutrition. Basel, Karger.

Indian Journal of Animal Sciences 73 (10): 1111-1113, October 2003

Enterococcal antagonism against food-borne pathogens

RANDHIR SINGH[1] and S R GARG[2]

CCS Haryana Agricultural University, Hisar, Haryana 125 004 India

Received: 21 October 2002; Accepted: 15 June 2003

ABSTRACT

Ninety isolates of *Enterococcus* spp. comprising 50 isolates of *E. faecalis*, 9 *E. gallinarum*, 17 *E. raffinosus* and 7 each of *E. pseudoavium* and *E. solitarius* obtained from the meat, fish, milk, sewage and excreta were examined for antagonistic activity against 2 isolates each of *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* Enteritidis by deferred antagonism method. Thirty-three isolates revealed inhibitory action on both the strains of *L. monocytogenes*. Two isolates of *Enterococcus gallinarum* inhibited *S. aureus* but none of the isolates inhibited *Salmonella* Enteritidis. It was concluded that enterococci had narrow spectrum of antagonistic activity

Key words: Antagonism, *Enterococcus*, Food-borne pathogens, *Listeria*, *Staphylococcus*, *Salmonella*

The genus *Enterococcus* is distributed widely in nature. It occurs in gastrointestinal tract of man and animals and also in the plants, soil and water. Enterococci also constitute natural microflora of foods including milk, dairy products, meat, fish and vegetables (Jay 1992). *Enterococcus* spp. have been reported to exhibit antagonism against a variety of pathogenic and spoilage organisms which has received attention of the scientific community worldwide as a potential alternative to chemical preservatives to control undesirable microorganisms in foods.

McKay (1990) reported antimicrobial activity of *E. faecium* against *Listeria innocua*. Arihara *et al.* (1991) demonstrated strong antagonism of *E. faecalis* on *L. monocytogenes*. Ben Embarek *et al.* (1994) recorded inhibitory potential of *E. faecium* on pathogenic and spoilage organisms including *L. monocytogenes, Clostridium* spp., *Lactococcus lactis* and *Shewanella putrefaciens*.

Several laboratories in different countries are currently engaged in the isolation of suitable strains of enterococci with an eye on their potential in food preservation (Olasupo *et al.* 1994, Laukova *et al.* 1999). The present studies were carried out to examine the strains of enterococci isolated from animals, animal products and other sources in the local environment for antagonistic properties against some major food-borne pathogens such as *L. monocytogenes, S. aureus* and *Salmonella* Enteritidis.

Present address: [1]Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Sciences, Punjab Agricultural University, Ludhiana 141 001.
[2]Associate Professor, Department of Veterinary Public Health, College of Veterinary Sciences.

MATERIALS AND METHODS

Enterococcus isolates

Isolates (90) of enterococci obtained from meat, fish, milk, sewage and excreta were examined for antagonism. These included 50 isolates of *E. faecalis*, 9 *E. gallinarum*, 17 *E. raffinosus* and 7 each of *E. pseudoavium* and *E. solitarius*.

Indicator bacteria

Two isolates each of *L. monocytogenes, S. aureus* and *Salmonella* Enteritidis were used as indicator organisms. Strains MTCC 1143 and MTCC 657 of *L. monocytogenes* were obtained from the Institute of Microbial Technology, Chandigarh. Isolates of *S. aureus* and *Salmonella* Enteritidis were taken from the culture collection of the laboratory.

Inhibition of indicator bacteria

Inhibitory activity was examined on tryptic soya agar supplemented with 0.6% yeast extract (TSAYE) by deferred antagonism method (Tagg *et al.* 1976). In this, 5 ml volumes of overnight broth cultures of test isolates of *Enterococcus* spp. were spotted on the surface of TSAYE plates. The plates were then incubated for 18-20 hr at 37°C to allow colony development. A thin layer of 3-4 ml semisolid TSAYE (containing 0.7% agar w/v) inoculated with 10^6 cells of indicator organism was overlaid over the surface of the incubated plate taking care not to disturb the spots of test isolates. After solidification of the medium, the plates were incubated again at 37°C for 18-20 hr. The plates were then examined for zones of inhibition of growth of indicator organism around the spots. Any clear zone around the test spots was considered antagonistic activity of the test culture

on the indicator organism.

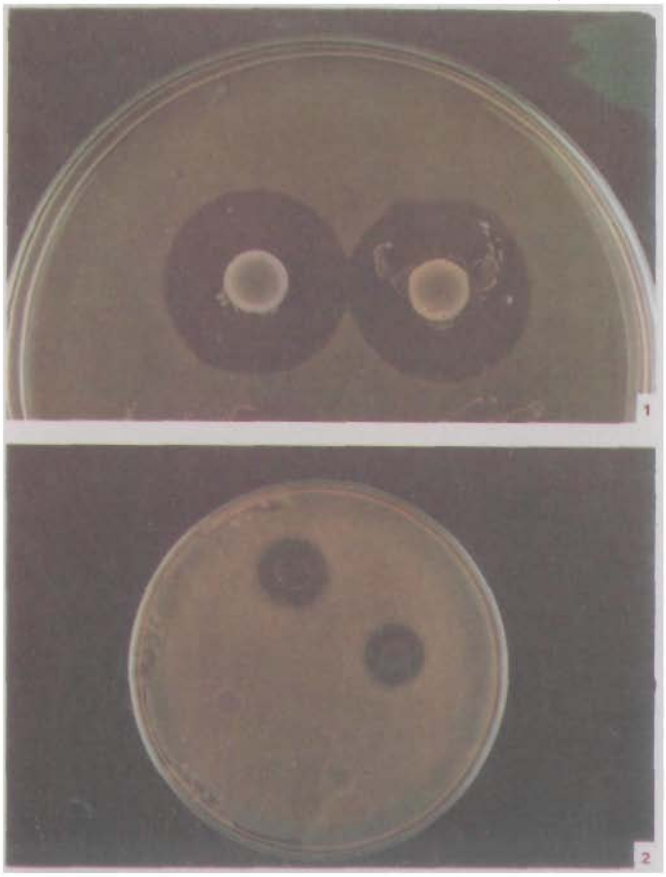
RESULTS AND DISCUSSION

Among 90 isolates of different *Enterococcus* spp. examined during the study, 33 (37%) revealed inhibitory action on both the indicator strains of *L. monocytogenes* (Table 1). These included 21 isolates (out of 50, 42%) of *E. faecalis*, 1 (out of 9, 11%) of *E. gallinarum*, 7 (out of 7, 100%) of *E. pseudoavium*, 3 (out of 17, 18%) of *E. raffinosus* and 1 (out of 7, 14%) of *E. solitarius*. The zones of inhibition by the isolates varied from 8 mm to 25 mm in diameter. While several isolates showed wide zones of inhibition (17-25 mm) around the test culture spots, others showed moderate (13-16 mm) or narrow (8-12 mm) zones of activity (Table 2, Fig. 1).

The antagonistic action of *Enterococcus* isolates on the 2 indicator strains of *L. monocytogenes* did not differ much. Same set of 33 isolates showed inhibition of both the indicator strains and, in general, the zones of inhibition were also similar, barring few exceptions in which the difference in diameters of clear zones was more than 3 mm.

Table 1. Inhibitory action of *Enterococcus* spp. on *L. monocytogenes* strain MTCC 1143 and MTCC 657

Species	No. of isolates showing inhibition / total no. of isolates								
	Chevon	Chicken meat	Fish	Raw milk	Sewage	Pig faeces	Buffalo faeces	Human faeces	Total
E. faecalis	4/4	6/11	4/8	2/6	1/6	3/12	0/1	1/2	21/50
E. gallinarum	1/2	0/3	-	-	0/4	-	-	-	1/9
E. pseudoavium	2/2	3/3	2/2	-	-	-	-	-	7/7
E. raffinosus	2/3	-	1/5	-	0/3	0/6	-	-	3/17
E. solitarius	-	0/1	1/4	0/1	-	0/1	-	-	1/7
Total	9/11	9/18	8/19	2/7	1/13	3/19	0/1	1/2	33/90



Figs 1-2. **1**. The zones of inhibition varied from 0.5 to 2.5 mm in diameter. **2**. The 2 isolates of *E. gallinarum* showed zones of inhibition varying from 17 mm and 20 mm in diameter.

These observations find support from the work of Arihara *et al.* (1991) who also reported 2 of the 6 *E. faecalis* isolates showing strong antagonism against 5 strains of *L. monocytogenes*.

Two isolates of *E. gallinarum* out of total 90 isolates of *Enterococcus* spp. caused inhibition of 2 strains of *S. aureus*. Both the isolates originated from sewage. Their zones of inhibition were 17 mm and 20 mm in diameter (Fig. 2). The isolates causing inhibition of *S. aureus* did not inhibit any of the two strains of *L. monocytogenes*. Olasupo *et al.* (1994) also isolated a strain of *E. faecium* from a Nigerian dairy product, which was antagonistic towards *L. monocytogenes*, other *Listeria* spp., *Lactobacillus* strains and *Enterococcus* strains, but not against *S. aureus*.

None of the 90 isolates of *Enterococcus* spp. showed inhibition of any of the two strains of *Salmonella* Enteritidis.

The isolates *E. faecium* P13 (Cintas *et al.* 1997) and *E. faecium* Cal 1 (Dutta *et al.* 1999) also did not inhibit *Salmonella* Typhimurium strain, which supports the present findings. However, Laukova *et al.* (1998) reported antagonistic activity of bacteriocin-like substance produced by *E. faecalis* V24 against *Salmonella* spp.

Enterocinogenic activity in the cell-free filtrates of enterococci was reported by several workers. Du Toit *et al.* (2000) reported that 7 out of 92 *Enterococcus* isolates from pig faeces were positive for enterocin production. Of these, 3 isolates of *E. faecium* showed broad spectrum activity against *Listeria* spp., *Clostridium* spp., *Enterococcus* spp., *Propionibacterium* spp. while 4 isolates of *E. faecalis* acted mainly against other *Enterococcus* spp. only. An enterocin with broad spectrum activity was also reported by Laukova *et al.* (1998, 2000). The enterocin from *E. faecalis* V24 of cattle dung origin was active against *L. monocytogenes, S. aureus, S. xylosus, Enterococcus* spp., *Enterobacter* spp..

Table 2. Inhibitory zones of *Enterococcus* spp. against *L. monocytogenes*

Species	Indicator strain	No. of isolates showing inhibition zone*				
		8-12 mm	13-16 mm	17-20 mm	21-25 mm	Total
E. faecalis	1143	12	6	2	1	21
	657	13	6	2	-	21
E. gallinarum	1143	1	-	-	-	1
	657	1	-	-	-	1
E. pseudoavium	1143	2	5	-	-	7
	657	3	4	-	-	7
E. raffinosus	1143	1	1	1	-	3
	657	2	-	1	-	3
E. solitarius	1143	1	-	-	-	1
	657	-	1	-	-	1
Total	1143	17	12	3	1	33
	657	19	11	3	0	33

* Zone includes test culture spots of 7 mm diameter.

Proteus spp., *Salmonella* spp. and *Y. enterocolitica*. Ohmomo *et al.* (2000) isolated *E. faecium* NIAI 157 from silage and its enterocin was also inhibitory to *L. monocytogenes*, *Enterococcus* strains, *Lactobacillus* strains, *Pediococcus* strains but not against *S. aureus*, *E. coli* and *Klebsiella pneumoniae*. Jennes *et al.* (2000) found a strain of *E. gallinarum* isolated from the intestine of ostrich to be enterocinogenic. The cell-free filtrate of the isolate inhibited the growth of *L. innocua*, *C. perfringens*, *P. aeruginosa*, *Salmonella* Typhimurium, *Propionibacterium*, *Lactobacillus* and *E. faecalis*.

The studies, thus, revealed that enterococci examined in the present investigations inhibited the growth of Gram-positive organisms, namely, *L. monocytogenes* and *S. aureus* but did not cause inhibition of *Salmonella* Enteritidis, a Gram-negative organism. Moreover, the inhibitory action against *L. monocytogenes* and *S. aureus* strains was shown by different sets of *Enterococcus* isolates. This suggested that enterococci had narrow spectrum of antagonistic activity.

Antagonistic activity revealed by the strains of enterococci in the present studies indicated the possibility of occurrence of enterocinogenic strains in the local environment which may have good potential for use as natural food preservatives and antimicrobial agents.

REFERENCES

Arihara K, Ogihara S, Sakata J, Itoh M and Kondo Y. 1991. Antimicrobial activity of *Enterococcus faecalis* against *Listeria monocytogenes*. *Letters in Applied Microbiology* **13**: 190-92.

Ben Embarek P K, Jeppesen V F and Huss H H. 1994. Antibacterial potential of *Enterococcus faecium* strain isolated from sour-vide cooked fish fillets. *Food Microbiology* **11**: 525-36.

Cintas L M, Casaus P, Havarstein L S, Hernandez P E and Nes I F. 1997. Biochemical and genetic characterization of enterocin P, a novel sec-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. *Applied and Environmental Microbiology* **63**: 4321-30.

Du Toit M, Franz C M A P, Dicks L M T and Holzapfel W H. 2000. Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. *Journal of Applied Microbiology* **88**: 482-94.

Dutta S, Chakraborty P and Ray B. 1999. Production, purification and characterisation of enterocin cal 1, a bacteriocin of *Enterococcus faecium* cal 1, isolated from goat meat. *Indian Journal of Microbiology* **39**: 15-22.

Jay J M. 1992. *Modern Food Microbiology*. 4th edn. Chapman and Hall, Inc., New York.

Jennes W, Dicks L M T and Verwoerd D J. 2000. Enterocin 012, a bacteriocin produced by *Enterococcus gallinarum* isolated from the intestinal tract of ostrich. *Journal of Applied Microbiology* **88**: 349-57.

Laukova A, Czikkova S, Dobransky T and Burdova O. 1999. Inhibition of *Listeria monocytogenes* and *Staphylococcus aureus* by enterocin CCM 4231 in milk products. *Food Microbiology* **16**: 93-99.

Laukova A, Czikkova S, Vasilkova Z, Juris P and Marekova M. 1998. Occurrence of bacteriocin production among environmental enterococci. *Letters in Applied Microbiology* **27**: 178-82.

Laukova A, Juris P, Vasilkova Z and Papajova I. 2000. Treatment of sanitary-important bacteria by bacteriocin substance V 24 in cattle dung water. *Letters in Applied Microbiology* **30**: 402-05.

McKay A M. 1990. Antimicrobial activity of *Enterococcus faecium* against *Listeria* spp. *Letters in Applied Microbiology* **11**: 15-17.

Ohmomo S, Murata S, Katayama N, Nitisinprasart S, Kobayashi M, Nakajima T, Yajima M and Nakanishi K. 2000. Purification and some characteristics of enterocin ON-157, a bacteriocin produced by *Enterococcus faecium* NIAI 157. *Journal of Applied Microbiology* **88**: 81-89.

Olasupo N A, Schillinger U, Franz C M A P and Holzapfel W H. 1994. Bacteriocin production by *Enterococcus faecium* NA 01 from 'wara'-a fermented skimmed cow milk product from West Africa. *Letters in Applied Microbiology* **19**: 438-41.

Tagg J R, Dajani A S and Wannamaker L W. 1976. Bacteriocin of Gram-positive bacteria. *Bacteriological Reviews* **40**: 722-56.

Indian Journal of Animal Sciences **73** (10): 1114-1117, October 2003

Antibacterial activity of buffalo polymorphonuclear cationic peptides on *Pasteurella multocida*

S C ROY[1] and T MORE[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 9 October 2002; Accepted: 9 June 2003

Key words: Antibacterial activity, Blood, Buffalo, Cationic peptides, *Pasteurella multocida*, Polymorphonuclear cells

Polymorphonuclear cells (PMNs) are important effector cells against most microbes. They kill the invaders by 2 distinct mechanisms; oxidative and non-oxidative. The oxidative mechanism involves the production of powerful oxidants (reactive nitrogen and oxygen intermediates) damaging the membrane of microbes, and the non-oxidative mechanism involves the degranulation of toxic microbicidal substances (cationic proteins and peptides) into the phagocytic vacuoles where the microbes are destroyed ultimately. Bowman (1995) studied role and importance of these peptide antibiotics in innate immunity. However, these mechanisms were reported to be ineffective in pasteurellosis of avian species (Truscott and Hirsh 1988). Pasteurellosis, among all the infectious diseases in India, is responsible for the highest mortality in buffaloes and cattle (Dutta *et al.* 1990) and is caused by *Pasteurella multocida* serotype B: 2. But, in buffaloes, the status of this PMN-mediated defence mechanism against *P. multocida* is not clear. In the present study an assessment of the non-oxidative microbicidal system of buffalo PMNs against *P. multocida* was attempted.

Healthy 3-year-old male Murrah buffaloes (4) were maintained under identical conditions. Peripheral blood was collected from the jugular vein of these buffaloes using the disodium salt of EDTA (1mg/ml) as an anticoagulant and used for separating PMNs. A pure culture of *P. multocida* serotype B: 2 strain P_{52} was obtained from the culture collection unit of the Standardization Division of the Institute. The cells were isolated from the buffalo blood as described previously (Birnboim and Kanabus-Kaminska 1985). The isolation process was completed within 2hr and the viability of the cells was assessed by the trypan blue exclusion technique. The purity of the preparations was also assessed by Leishmann's staining. The adopted isolation procedure gave an overall recovery of 70% of the PMNs present in the blood. Viability and purity of the cells was 90 and 92% respectively.

Present address: [1]Ph. D. Scholar (In-Service), Division of Animal Biochemistry, NDRI, 132 001.

[2]Principal Scientist, Division of Biochemistry.

Granular proteins and peptides were extracted as described by Gennaro *et al.* (1983b) with some modifications. Briefly, 7.5×10^8 PMNs were suspended in 5ml of 0.34M sucrose in PBS, pH 7.4, containing 2 mmol/L phenyl methyl sulfonyl fluoride (PMSF) and 2 mmol/L EDTA. The mixture was subjected to restricted sonication (4 cycles at 8μ amplitude for 30s each) in an ultrasonic disintegrator under ice-cold conditions. The granule-rich supernatants were combined and centrifuged at 27,000x g for 30 min at 4° C in a Sorvall RC-5B refrigerated superspeed centrifuge (Du Point Instruments Co., USA). The supernatant was discarded and the greenish-white pellet of granules was acid extracted at 4° C for 4hr in 10 volumes of 0.2 mol/L sodium acetate, pH 4.0, while stirring using a magnetic stirrer. The extract was recentrifuged at 27,000x g for 30 min and reextracted with 5 volumes of the same buffer. The extracts of the granules were pooled and subsequently dialyzed against large volumes of 0.2 mol/L NaCl in 10mmol/L sodium phosphate, pH 7.0 in dialysis tubing of a molecular weight cut off of 2.5 kilo Dalton (KDa), with a recovery of protein close to 100%. Subsequently, the pooled, dialyzed extract was subjected to preparative acid urea polyacrylamide gel electrophoresis (AU-PAGE) (Selsted *et al.* 1984) to separate cationic peptides from crude proteins. The preparative AU-PAGE was able to yield 0.45 mg of peptides from 5 mg of crude proteins. The protein was stored at –20° C until further use. Protein concentration through out the experiment was estimated by the method of Lowry *et al.* (1951).

In the first experiment, the bacterial uptake of granular peptides by *P. multocida* was assessed. *P. multocida* serotype B: 2 strain P_{52} was grown in brain heart infusion broth at 37° C for 24hr. The cultures were centrifuged at 10,000x g for 15 min. The pellet obtained was washed twice and suspended in PBS toA an optical density of 0.500 at 540 nm representing approximately 2×10^9 bacteria/ml. 200 mg of PMN granular cationic peptides obtained above was incubated at 37° C for 30 min with 1×10^9 CFU of *P. multocida* in 200 ml of PBS. After incubation, the assay mixtures were centrifuged at 10000x g for 15 min and the supernatants

obtained were recentrifuged under the same conditions. The final supernatants were then subjected to analytical AU-PAGE as per Panym and Chalkley (1969). However, electrophoresis was performed by applying a constant current of 2 mA/gel slot. The aim of this experiment was to obtain a preliminary idea on the nature of interaction between the peptides and bacteria. Uptake of cationic peptides by *P. multocida* was observed by analytical AU-PAGE. *P. multocida* removed to a considerable extent some of the cationic peptides as evidenced by disappearance of 6 most cathodic peptide bands and diminishing of remaining 9 major bands in the gel i.e. by comparing the AU-PAGE of before (Lane A) and after the peptides exposure to bacteria (Lane B) (Fig.1). The separated peptides when subjected to analytical AU-PAGE, showed as many as 15 peptide bands using Coomassie brilliant blue-R and suggested that many of the low molecular weight peptides which could not be detected in preparative AU-PAGE using eosin Y could only be detected with Coomassie brilliant blue-R.

In the second experiment, the stock cultures of *P. multocida* serotype B: 2 strain P52 were first subcultured in (Brain heart infusion broth) BHIB at 37° C for 24hr. 1 ml of diluted culture (1: 10 in PBS, pH 7.4) was poured on the blood agar in a petri-dish and it was kept in an incubator at 37° C for 30 min. Filter paper disks impregnated with different concentrations of cationic peptides (0.2μg, 0.5μg, 1μg and 1.5μg) and crude

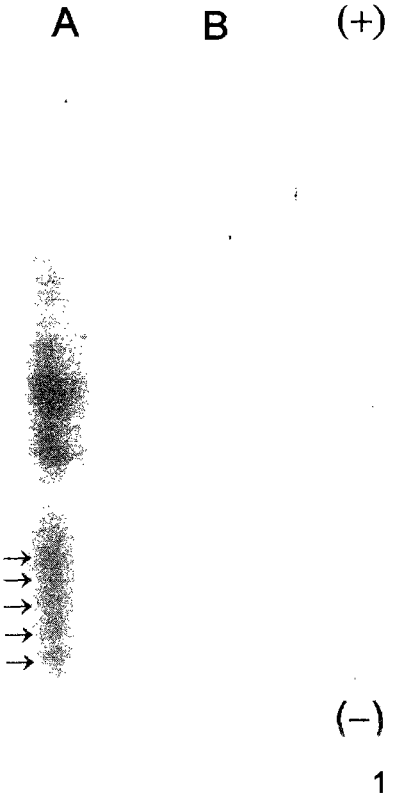
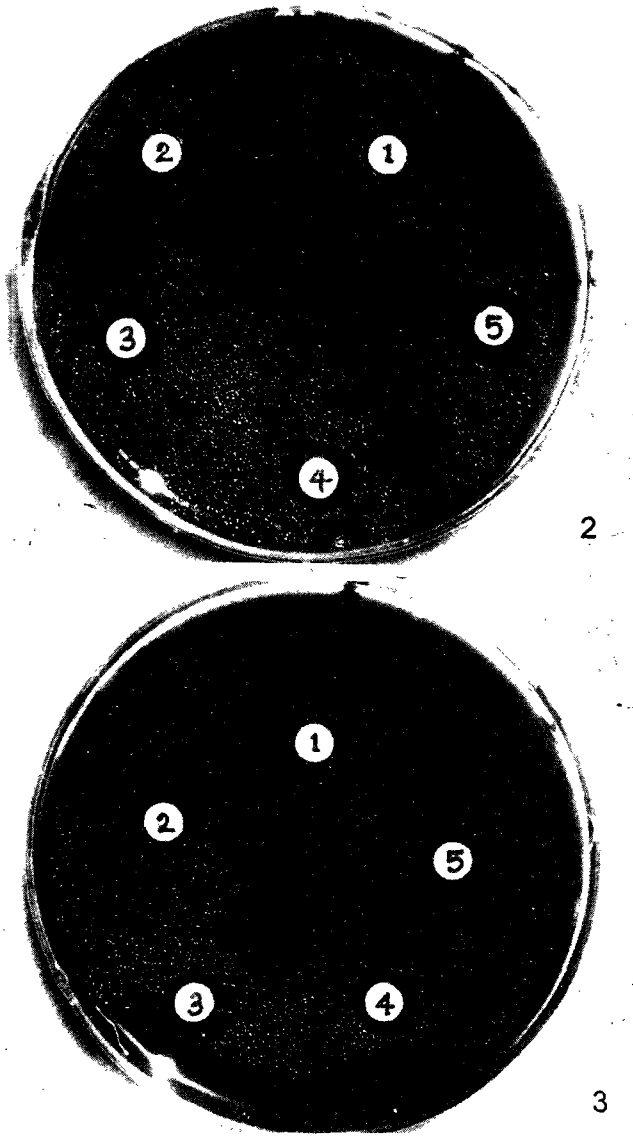


Fig 1. Bacterial uptake of cationic polypeptides of buffalo PMN. Lanes: A, cationic peptides; B, cationic peptides plus *P. multocida*. Arrows shows that peptides present in Lane A are not present in Lane B.



Figs 2-3. 2. *P. multocida* growth inhibition by cationic peptides of buffalo PMN granules. Disk-1, control (PBS); Disk-2, 0.2 μg; Disk-3, 0.5 μg; Disk-4, 1 μg and Disk-5, 1.5 μg of cationic peptides. 3. *P. multocida* growth inhibition by cationic proteins of buffalo PMN granules. Disk-1, control (PBS); Disk-2, 3.6 μg; Disk-3, 9 μg; Disk-4, 18 μg and Disk-5, 27 μg of cationic proteins.

acid extracted proteins (3.6μg, 9μg, 18μg and 27μg) were applied on the medium. A control disc was also applied after soaking it in PBS. The petri-dish was incubated at 37° C for 24 hr and the bacterial colonies were observed. In this test, an optimum antibacterial activity against *P. multocida* with 0.5 μg of separated cationic peptides was noticed (Fig.2), whereas, 9 μg of whole crude acid extracted proteins were required to get the same results (Fig.3).

The non-oxidative defence mechanisms are based on microbicidal proteins and peptides residing within the cytoplasmic granules in the PMNs. Many of these proteins and peptides have been identified and characterized in human,

guinea pig, rabbit, and cattle (Gennaro *et al.* 1983b, Selsted *et al.* 1984, Ganz *et al.* 1985, Lehrer *et al.* 1985, Selsted *et al.* 1985, Lichenstein *et al.* 1986, Rice *et al.* 1987). These proteins and peptides are myeloperoxidase, lactoferrin, elastase, lysozyme and natural antibiotic defensins and bactenecins, which differ in size and charge and in the range of their antimicrobial activity. Informations on these proteins and peptides in ruminants are limited, although a few reports are available for *Bos taurus* (Gennaro *et al.* 1978, 1983a, 1989). No report is available on O_2-independent microbicidal system of buffalo PMNs against *P. multocida*, the bacteria of clinically relevance.

The present study revealed that the incubation of buffalo PMN cationic peptides with *P. multocida* caused a selective uptake of peptides by the bacteria as is evidenced by disappearance and diminishing of certain peptide bands in AU-PAGE of supernatant proteins obtained after incubation with bacteria (Fig.1). Gennaro *et al.* (1983b) reported similar results taking *Staphylococcus aureus* as test bacteria. They are of the opinion that the disappearance of the peptide bands in AU-PAGE analysis may be attributed to selective adsorption or internalization (or both) of peptides by bacteria. However, it is argued that membrane damage is the major reason of antibacterial mechanism of the peptides (Lehrer *et al.* 1989). Membranes were early recognized as the targets for many antibacterial peptides and artificial membranes have been used to demonstrate voltage-dependant channel formation with defensins (Kagan *et al.* 1990). Selsted *et al.* (1993) reported 13 structurally homologous peptides from a granule-rich cytoplasmic fraction of bovine blood neutrophils. These peptides are characterized by a highly cationic 38-42-residue amino acid chain, which includes 6 invariably spaced cysteins that form 3 disulfide bonds, and were named as ß-defensins. These are different from α-defensins of Ganz *et al.* (1985).

It was reported earlier from this laboratory that buffalo PMN contained at least 11 cationic proteins and peptides. Three peptides separated by gel permeation chromatography were antibacterial against *Escherichia coli* and *Staphylococcus aureus* (Sarmah *et al.* 1993a; Sarmah and More 1993b). The present study that involved the separation of peptides by preparative AU-PAGE revealed that growth of *P. multocida* is arrested in the presence of both cationic peptides and acid extracted crude proteins. This was evident from transparent circular inhibition zones of varying diameters at sites of filter disks impregnated with cationic peptides and crude proteins. Similar results were reported by Gennaro *et al.* (1983b) against *S. aureus* and *E. coli*. The present evidence suggested that the antibacterial activity is largely due to cationic nature of these proteins and peptides against *P. multocida*.

SUMMARY

In this study, our observations suggested that bacterial incorporation of cationic peptides formed one of the bases of antibacterial activity of PMNs. These studies with the partially purified peptides yielded a valuable insight into the potential antimicrobial constituents of PMNs against *P. multocida*.

ACKNOWLEDGEMENT

We thank Director of the Institute, and Head of the Division, for providing necessary research facilities.

REFERENCES

Birnboim H C and Kanabus-kaminska M. 1985. The production of DNA strand breaks in human leukocytes by superoxide anion may involve a metabolic process. *Proceedings of the National Academy of Sciences of the USA* **82**: 6820-6824.

Bowman H G. 1995. Peptide antibiotics and their role in innate immunity. *Annual Review of Immunology* **13**: 61-92.

Dutta J, Rathore B S, Mullick S G, Singh R and Sharma G C. 1990. Epidemiological studies on occurrence of haemorrhagic septicaemia in India. *Indian Veterinary Journal* **67**: 893-99.

Ganz T, Selsted M E, Szklarek D, Harwig S S L, Daher K, Bainton D F and Lehrer R I. 1985. Defensins: natural peptide antibiotics of human neutrophils. *Journal of Clinical Investigation* **76**: 1427-35.

Gennaro R, Schneider C, De nicola G, Cian F and Romeo D. 1978. Biochemical properties of bovine granulocytes. *Proceedings of the Society of Experimental Biology and Medicine* **157**: 342-47.

Gennaro R, Dewald B, Horishberger U, Gubler H U and Baggiolini M. 1983a. A novel type of cytoplasmic granule in bovine neutrophils. *Journal of Cell Biology* **96**: 1651-61.

Gennaro R, Dolzani L and Romeo D. 1983b. Potency of bactericidal proteins purified from the large granules of bovine neutrophils. *Infection and Immunity* **40**: 684-90.

Gennaro R, Skerlavaj B and Romeo D. 1989. Purification, composition and activity of two bactenecins, antibacterial peptides of bovine neutrophils. *Infection and Immunity* **57**: 3142-46.

Kagan B L, Selsted M E, Ganz T and Lehrer R I. 1990. Antimicrobial defensin peptides from voltage-dependant ion-permeable channels in planar lipid bilayer membranes. *Proceedings of the National Academy of Sciences of the USA* **87**: 210-14.

Lehrer R I, Daher K, Ganz T and Selsted M E. 1985. Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes. *Journal of Virology* **54**: 467-72.

Lehrer R I, Barton A, Daher K A, Harwig S S L, Ganz T and Selsted M E. 1989. Interaction of human defensins with *Escherichia coli*: mechanism of bactericidal activity. *Journal of Clinical Investigation* **84**: 553-61.

Lichtenstein A, Ganz T, Selsted M E and Lehrer R I. 1986. *In vitro* tumor cell cytolysis mediated by peptide defensins of human and rabbit granulocytes. *Blood* **68**: 1407-10.

Lowry O H, Rosenbrough N J, Farr A L and Randall R J. 1951. Ptotein measurement with the folin-phenol reagent. *Journal of Biological Chemistry* **193**: 265-75.

Panyim S and Chalkley R. 1969. High resolution acrylamide gel electrophoresis of histones. *Archives of Biochemistry and Biophysics* **130**: 337-46.

Rice W G, Ganz T, Kinkade J M Jr, Selsted M E, Lehrer R I and

Parmley R T. 1987. Defensin-rich dense granules of human neutrophils. *Blood* **70**: 757-65.

Sarmah S, Reddy G R and More T. 1993a. Cationic antibiotic granular peptides of buffalo polymorphonuclear cells. *Indian Journal of Animal Sciences* **63**: 618-22.

Sarmah S and More T. 1993b. Evidence for homology of buffalo and goat granular cationic peptides. *Comparative Immunology, Microbiology and Infectious Diseases* **19**: 47-53.

Selsted M E, Szklarek D and Lehrer R I. 1984. Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes. *Infection and Immunity* **45**: 150-54.

Selsted M E, Harwig S S L, Ganz T, Schilling J W and Lehrer R I. 1985. Primary structures of three human neutrophil defensins. *Journal of Clinical Investigation* **76**: 1436-39.

Selsted M E, Tang Y Q, Morris W L, Mcguire P A, Novotny M J, Smith W, Henschen A H and Cullor J S. 1993. Purification, primary structures and antibacterial activities of ß-defensins, a new family of antimicrobial peptides from bovine neutrophils. *Journal of Biological Chemistry* **268**: 6641-48.

Truscott W M and Hirsh D C. 1988. Demonstration of an outer membrane protein with antiphagocytic activity from *Pasteurella multocida* of avian origin. *Infection and Immunity* **56**: 1538-44.

Indian Journal of Animal Sciences **73** (10): 1118-1119, October 2003

Induced coccidiosis in goats*

M J MAHESH KUMAR[1] and G A BALASUBRAMANIAN[2]

Veterinary College and Research Institute, Namakkal, Tamil Nadu 637 001 India

Received:5 December 2001; Accepted: 11 June 2003

Key words: Coccidiosis, Goats

Coccidiosis is one of the most economically important diseases of goats world over and outbreaks of the disease have caused heavy mortalities (Chhabra and Pandey 1991, 1992). Farmers face economic loss due to decrease in bodyweight and reduction in milk yield (Bhatia 1976). It causes more damage to the young stock but the adult animals suffer from a chronic infection without evincing any clinical symptoms and shed oocyst in small number thus act as carriers of infections to young ones (Gill and Katiyar 1961). Hence, in this study attempt was made to determine the effect of mixed coccidial infection predominantly *Eimeria arloingi*, *E. ninakohlyakimovae, E. christenseni* in 2-3 months old kids as under field condition invariably mixed infection occurs.

Goat kids (14), 2-3 month -old, were maintained under intensive system of management in which 8 animals were included in infected group and 6 as control. These were provided *ad lib.* standard feed and water throughout the experiment. Before starting the experiment, both the groups were acclimatized for 7 days during which animals were screened for coccidiosis. The animals of treatment group were administered 3 doses of dexamethasone sodium (25 mg/kg b. wt) intramuscularly on alternate day before the experiment to suppress their immune status. Subsequently on day 0 these were infected with 2.5 lakhs sporulated oocyst of mixed species predominantly *E. arloingi, E. ninakohlyakimovae, E. christenseni* by oral drenching. Control animals received 2 ml of distilled water on day 0. Infected kids were sacrificed at 4 days interval from 4 to 28 day post inoculations (DPI).

Blood samples were collected aseptically from jugular vein of both control and infected group using EDTA as anticoagulant at 4 days interval from 4 to 28 DPI. Haematological study included determination of haemoglobin (Hb) by microhaematocrit method, total erythrocyte count (TEC) using Hayems fluid and total leucocyte count (TLC) using Thomas fluid. The results were statistically analysed as per Snedecor and Cochran (1989).

Kids suffering from coccidiosis exhibited the symptoms of reduced feed intake, abdominal pain, bleating and foul smelling diarrhoeic faeces containing streaks of blood with mucus and soiled hind quarters at 12 DPI. At 20 DPI tenesmus with abdminal pain, anemia and severe dehydration were recorded and generalized incordination and grinding of teeth during 24 to 28 DPI. These observations were in concurrence with the earlier findings of Gill and Katiyar (1961), Lima (1981), Aumont *et al.* (1986) and Dash *et al.* (1991).

Comparison of Hb, PCV, TEC values of both control and infected groups showed highly significant ($P<0.01$) decrease in infected group during 12 to 28 DPI indicating anaemia but reduction in the values was not significant during 4 to 8 DPI (Table 1). Similar observations were recorded by Rama *et al.* (1978), Aumont *et al.* (1986) and Hayat *et al.* (1990)

Dharmendra Kumar and Hafeez (1999) attributed the low haematological value in infected lambs to intestinal tissue damage and haemorrhagic enteritis followed by blood loss due to endogenous stages of eimerican parasites. However, Rama *et al.* (1978) and Smith and Skerman (1994) observed an increase in PCV due to haemoconcentration associated with dehydration.

The TLC values of infected group showed significant increase ($P<0.01$) between 12 to 28 DPI and supported the observation of Rama *et al.* (1978). Smith and Sherman (1994) attributed the higher TLC value in coccidiosis infected goats due to extensive damage of the intestinal mucosa. The present study has highlighted that clinical signs and haematological alternations are more pronounced during 12 to 28 DPI in coccidiosis.

SUMMARY

Reduced feed intake, tenesmus with abdominal pain, anaemia and foul smelling diarrhoea containing streaks of blood with mucus were recorded. A significant decrease ($P<0.01$) in the value of Hb, PCV, TEC and significant

Part of M.V. Sc. Thesis submitted to Tamil Nadu Veterinary and Animal Science University, Chennai 600 051.

Present address: [1]Technical Officer, Small Animal Facility, National Institute of Immunology, New Delhi 110 067.

[2]Associate Professor and Head, Department of Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu.

Table 1. Effect of *Eimeria* infection on mean (±SE) values of Hb, PCV, TEC and TLC

Day post inoculation	Haemoglobin (g per cent)		Packed cell-volume (percentage)		Total erythrocyte count (m/cmm)		Total leucocyte count (thousand/cmm)	
	Control	Infected	Control	Infected	Control	Infected	Control	infected
4 DPI	9.85±0.19	9.25^{b}±0.20	32.50±1.09	31.43^{c}±0.66	11.48±0.22	10.8^{b}±0.10	9.50±0.09	9.61^{a}±0.08
8DPI	9.53±0.21	8.87^{b}±0.23	32.50±1.41	28.42^{bc}±1.63	11.53±0.62	9.93^{b}±0.35	10.02±0.59	11.34^{ab}±0.82
12 DPI	9.78±0.10	7.71^{a}±0.14	29.50±1.26	25.00^{ab}±0.73	11.93±0.34	8.08^{a}±0.10	9.16±0.20	11.95^{b}±0.66
16 DPI	9.55±0.08	7.30^{a}±0.17	28.66±0.80	22.60^{a}±1.03	12.17±0.32	7.92^{a}±0.19	8.92±0.40	12.01^{b}±0.41
20 DPI	9.75±0.10	7.20^{a}±0.18	28.75±0.87	22.37^{a}±0.97	12.45±0.26	7.85^{a}±0.13	8.90±0.13	12.13^{b}±0.71
24 DPI	9.85±0.08	7.10^{a}±0.11	29.29±0.18	22.41^{a}±0.74	12.73±0.24	7.76^{a}±0.08	8.96±0.19	12.43^{b}±0.28
28 DPI	9.93±0.10	7.60^{a}±0.20	29.00±0.73	23.00^{ab}±1.00	12.18±0.41	8.05^{a}±0.15	8.85±0.96	12.58^{ab}±0.07

Means bearing same superscript in the same column do not differ significantly (P<0.01).

increase (P<0.01) in the values of TLC were recorded from 12 to 28th day of the experiment indicating that in induced coccidiosis clinical expression become apparent only from 12th DPI to end of the experiment period.

ACKNOWLEDGEMENT

The authors thank the Dean, Veterinary College and Redearch Institute, Namakkal, Tamil Nadu, for providing necessary facilities to conduct the study.

REFERENCES

Aumont G, Yvore P and Nault A E S. 1986. Experimental coccidiosis in goats. 2. Effects of parasitism on nutritional balances and some blood parameters. *Ann Rech Vet* **17** (2) : 191-96.

Bhatia B B. 1976. Save lambs and kids from coccidiosis. *Indian Farming* **27** : 32-33.

Chhabra R C and Pandey V S. 1991. Prevalence of goat in Zimbabwe. *Veterinary Parasitology* **39**: 199-205.

Chhabra R C and Pandey V S. 1992. Prevalence of coccidiosis in sheep in Zimbabwe. *Small Ruminant Research* **8**: 257-64.

Dash B, Misra S C and Panda M R. 1991. A note on serum enzyme activity in experimental coccidiosis in kids. *Indian Veterinary Journal* **68** (2): 185-86.

Dharmendra Kumar B V and Hafeez M D. 1999. Efficacy of monensin and amprolium against sub clinical coccidiosis in lambs. *Indian Veterinary Journal* **76**: 965-67.

Gill B S and Katiyar R D. 1961. An acute outbreak of coccidiosis (*Eimeria arloingi*, Mortel, 1905) in kids of Orai sheep breeding farm. *Indian Journal of Veterinary Science and Animal Husbandry* **31**: 212-16.

Hayat C S, Malik A P, Anwar A and Iqbal Z. 1990. Effect of experimentally induced coccidiosis on some blood parameters and productivity of lambs. *Pakistan Veterinary Journal* **10** : 60-62.

Lima J D. 1981. Life cycle of *Eimeria christenseni* Levine, Ivens& Fritz, 1962 from the domestic goat (*capra hircus*). *Journal of Protozoology* **28** (1) : 59-64.

Rama S P, Singh C D N, Sinha B K and Prasad L N. 1978. Experimental coccidiosis in sheep: Haematological observation. *Indian Journal of Veterinary Science and Animal Husbandry* **48**(4) : 197-99.

Smith C M and Sherman M D. 1994. *Goat Medicine*. pp : 312-18. Lea and Febiger, Philadelphia.

Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 6th edn. Iowa state University. Ames, Iowa.

Indian Journal of Animal Sciences 73 (10): 1120-1122, October 2003

Some haematobiochemical studies on Garole sheep infected with amphistome parasites

K PUROHIT[1], M K BHOWMIK[2], S ROY[3], A S SINGH[4] and S K MUKHOPADHAYAY[5]

West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata, West Bengal 700 037 India

Received: 10 February 2003; Accepted: 5 June 2003

Key words: Haemato-biochemical changes, AST, ALT, Sheep, Amphistomiasis, Parasitology

The native place of Garole sheep is the saline belt of South 24-Parganas district of West Bengal specifically the Sundarban delta (4226 km^2). The animal is a highly prolific breed among the sheeps, their average adult body weight ranges 12-16 kg and considered as the real micro sheep of India (Sharma *et al.* 1999). The sheep has specific characteristics of adaptability to hot and humid climatic conditions, grazing in knee-deep water and high mothering instinct for their neonates. Field survey reports indicated that amphistomiasis and pneumonia are the major threat to this breed (Singh and Bohra 1996). Stomach flukes or conical flukes are widely prevalent in the Asian continent. Amphistomiasis in sheep is clinically manifested by persistent foetid diarrhoea with tenesmus, anorexia, rapid emaciation, oedematous swelling in sub-mandibular region, weakness, unthriftiness, depression, anaemia (Alwar 1949). Scanty information is available about the haematological and biochemical changes in Garole sheep infected with paramphistome. Therefore the present study was undertaken to report the some of important blood picture of Garole sheep infected with amphistome parasites.

Blood samples were collected by venipuncturing from the jugular vein of 10 heavily infected Garole sheeps age about 1½ years with a sterile 18 guage dry needle and a clean, prepackaged plastic syringe in the morning. For comparative study the blood samples were also taken from the equal number of healthy Garole sheep of similar age group and same locality as control group. Blood samples of both the groups of animals were collected in sterilized evacuated and labelled tubes. One batch of tubes containing dried dipotassium EDTA (@ 1 mg/ml) was used for haematological studies. A second batch tubes contained sodium floride (@ 1mg/ml) for glucose measurement. For serum separation, about 15 ml collected whole blood in the centrifuge tube was allowed to clot for about 30 min then rimmed with an applicator stick and centrifuged exactly at 2000 rpm for 10 min. The supernatant serum was carefully aspirated with the help of automatic microlit pipette and placed in the clean labeled plastic vials and preserved in deep freeze for further biochemical profiles assay. The blood samples were estimated as per standard prescribed methods like haemoglobin, PCV, TEC, TLC, DLC and platelet count (Schalm *et al.* 1986), blood glucose (Neison and Somogyi 1969). Serum total protein, albumin and globulin (Wooten and Freeman 1982), serum AST, and ALT (Reitman and Frankel 1957) and serum alkaline phosphatase (Kind and Kings 1954). The data pertaining to haematobiochemical profile were analysed for variance and tested for statistical significance employing Duncan multiple range test (Snedecor and Cochran 1994).

The infected animals had foetid diarrhoea, dysentry, edematous swelling, showing unthriftiness, anaemia and poor condition in body conformation followed by foetal examinations were confirmed for amphistomiasis. Haematological findings of both parasites and control Garole sheep groups are presented in Table 1. A significantaly pohaemoglobinaehymia, erythrocytopenia and low packed-cell volume (PCV) indicated anaemia in the amphistome-infected animals, which conformed with the reports of Lengy

Table 1. Mean haematological value of paramphistome infected and control groups of Garoel sheep

Parameters	Control group	Infected group
Haemoglobin (g%)*	11.17 ± 0.61^a	8.64 ± 0.72^b
PVC (%)**	36.78 ± 1.99^a	27.60 ± 2.28^b
TEC (10^6/cu.mm)*	11.11 ± 0.63^a	8.86 ± 0.64^b
TLC (10^3 cu.mm)**	10.64 ± 0.82^b	13.54 ± 0.56^a
Platelets (10^6 cu.mm)*	3.30 ± 0.51^a	1.86 ± 0.31^b
Differential leucocyte count		
Neutrophils (%)**	27.55 ± 1.86^a	15.18 ± 1.49^b
Eosinophils (%)**	4.02 ± 0.65^b	7.43 ± 0.89^a
Basophils (%)^{NS}	0.65 ± 0.13	0.76 ± 0.18
Lymphocytes (%)*	62.52 ± 2.20^b	69.83 ± 2.47^a
Monocytes (%)^{NS}	5.26 ± 0.53	6.80 ± 0.18

Mean values bearing different superscript in a row differ significantly.

** $P < 0.01$, * $P < 0.05$, NS, nonsignificant effect.

Present address: [1]Ph.D. Scholar, Department of Veterinary Pathology, [2]Director, DREF, [3]Professor, Department of Physiology, [4]Ph.D. Scholar, Department of Animal Nutrition, [5]Head, Department of Veterinary Pathology.

(1962). The development of anaemia might be due to presence of stomach flukes of family gastrothylocidae, which are recognized as active blood suckers in the rumen (Lemarire 1936). In parasitized sheep, anaemia might be resulted from chronic inflammatory reactions where iron was diverted to tissue and subsequently not available for the synthesis of haemoglobin. According to Misra *et al.* (1996) anaemia in the parasitized animals might also be due to sucking of blood by the immature paramphistomes and the haemorrhages caused due to their deep penetration into the mucosa and submucosa. In infected animals, there was significant ($P<0.01$) leucocytosis and similar findings were also reported by Misra *et al.* (1996). The result of leucocytosis was mainly contributed to the higher ($P<0.01$) numbers of lymphocytes and eosinophils. Lymphocytes play an important role in inflammation and immune response and their number in circulation indicated chronically of the disease and production of cell-mediated immunity (Schalm *et al.* 1986). The eosinophilia in the amphistome-infected sheep was also reported by Gill and Bali (1989). This is an indication of parasitism. The parasiticidal action of eosinophil is presence of antibody and/or complement is clearly established. The killing process is mediated primarily by major basis protein, peroxidase H_2O_2 - halide complex and oxygen metabolites liberated from the granules of the eosinophils (Vegad 1995). The parasitized animals had a significant ($P < 0.01$) neutropaenia which might have been caused by depression of haemopoietic system by the toxic products of the parasites. Monocyte and basophil showed non-significant variation (Table 1). Platelet count of the infected animals was lower ($P<0.05$) than that of the value of control sheep. Thrombocytopenia in the parasitized animal might also be due to sucking of blood by the immature paramphistome resulting in haemorrhage and inflammation but no literature is currently available to compare the findings of the present study.

Mean values of the blood biochemical profile of amphistome infested and control Garole sheep are given in Table 2. The mean total serum protein and albumin decreased significantly ($P<0.05$), whereas the globulin level increased in the infected animals than that in control sheep. These results are in agreement with the findings of Siddiqua *et al.* (1989). Serum protein level decreased may be due to the fact that volatile fatty acids were mostly utilized for supplying energy demand of the animal for maintenance and not being available for amination to form amino acid in the system. Hypoproteinaemia with simultaneous reduction of albumins to globulin ratio in the infected animals is likely due to marked distruction and disintegration of parenchymatous tissues. The decrease in albumin value is probably due to liver damages and increased catabolism of albumin through the gastrointestinal leakage. The inflammation and ulceration of the small instestine by immature flukes might also cause poor absorption of protein breakdown products resulting to low blood level of albumin. The rise of globulin value might be attributed to the response of body defence mechanism to the foreign body invasion. Globulin level increased was owing to increase level of Ig G-1 fraction, as also reported in fascioliasis (Movsesijan *et al.* 1974). Parasite infected Garole sheep had a significant ($P<0.05$) reduction of blood glucose level than that of control animals. This corroborated well with the observations of Lengy (1960). Pantelouris (1965) also reported that adult flukes consumed glucose and galactose. Hypoglycaemia might be due to depletion of liver glycogen resulting from liberation of toxic products by the worms. Serum alanine amino transferase (ALT) and asparate amino transferase (AST) were statistically higher (Table 2) in the infected sheep than that in control animals. Similar findings were also reported by Siddiqua *et al.* (1989). Enzyme activity increased in a wide range of inflammatory disease conditions particularly digestive, cardiac, urinary and arthritic diseases (Hawk 1965). There was also significant ($P<0.05$) increase in alkaline phosphatase in infected sheep than that in control animal. This increase level may be due to increase enzymatic activity associated with inflammatory changes.

Table 2. Mean blood biochemical values of paramphistome infected and control groups of Garole sheep

Parameters	Control group	Infected group
Total protein (g/dl)*	6.59 ± 0.40^a	5.55 ± 0.28^b
Albumin (g/dl)*	3.82 ± 0.50^a	2.30 ± 0.30^b
Globulin (g/dl)*	2.72 ± 0.22^b	3.58 ± 0.26^a
A: G ratio*	1.18 ± 0.17^a	0.65 ± 0.12^b
Glucose (g/dl)*	50.89 ± 1.92^a	43.01 ± 2.09^b
AST (I.U.)**	56.30 ± 1.88^b	76.49 ± 2.10^a
ALT, (I.U.)*	17.57 ± 1.42^b	26.79 ± 3.32^a
Alkaline phosphatase (I.U.)*	8.40 ± 0.28^b	9.48 ± 0.31^a

Mean values bearing different superscript in a row differ significantly, ** $P < 0.01$, * $P < 0.05$.

It may be inferred from this study that there was marked difference in the characteristic of haematobiochemical constituents in Garole sheep because of paramphistome infestation.

SUMMARY

An experiment was conducted to investigate the haemato-biochemical changes of Garole sheep infected with amphistome parasites in Sundarban delta, West Bengal. For the present study, the blood samples were collected from the jugular vein of 10 heavily amphistone infected animals about $1\frac{1}{2}$ years of age and also taken from equal number of healthy Garole sheep of the similar age group from the same locality as control group. The blood samples were analysed for some of important haemato-biochemical constituents like haemoglobin, TEC, TLC, platelet, neutrophil, eosinophil, lymphocyte, total protein, albumin, globulin, glucose, AST, ALT and alkaline phosphatase. The mean values of blood

constituents for amphistome infected and control groups showed significant differences due to severe parasitic infestation. It may be deduced from this finding that amphistone parasite infected Garole sheep marked difference in the characters of haemato-biological constituents.

ACKNOWLEDGEMENT

The senior author is highly grateful to Professor (Mrs) P Deb, Department of Veterinary Pathology, for her encouragement and keen interest to carry out this study.

REFERENCES

Alwar V S. 1949. Amphistomiasis-A review of the literature. *Indian Veterinary Journal* **25**: 417-24.

Gill J S and Bali H S. 1989. Haematology of sheep experimentally infected with *Paramphistomum cervi. International Journal of Animal Sciences* **4**: 171-173.

Hawk P B. 1965. *Physiological Chemistry.* 14th edn, pp. 1124 - 26. McGraw-Hill Book Co., London.

Kind P R N and King E J. 1954. Estimation of plasma phosphatase by determination of hydrolyzed phenol with aminoantipyrin. *Journal of Clinical Pathology.* 7: 332-34.

Lemarire N M. 1936. Traited helminthologic medicate et. *Veterinaria* Paris. *Fide Bullitens Epizootic Diseases of Africa* **12**: 439-54.

Lengy J. 1960. Bulletin. *Research Council Israel.* **9**: 71 - 130. *(fide Advances in Parasitiology* **9**: 53 - 59.)

Lengy J. 1962. *Refuah Veterinary.* **19**: 111-15. *(fide Advances in Parasitology* **9**: 53-59.)

Misra S C, Panda D M and Parida S. 1996. Haematological and histological alterations of immature paramphistomiasis in lambs. *Indian Veterinary Journal* **73**: 1274-76.

Movsesijan M, Jouanouic B, Aalund O and Nansen P. 1974. *Pathophysiology of Parasitic Infection.*pp. 34. Academic press. New York, San Fransisco, London.

Neison N and Somogyi M. 1969. Estimation of glucose. *Practical Clinical Biochemistry.* 4th edn, pp. 89-90. (Ed.) Varley M. Heineman practical Book Ltd, London.

Pantelouris E M. 1965. *Research in Veterinary Science* **6**: 330 - 33.

Reitman S and Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American Journal of Clinical Pathology* **28**: 55-63.

Schalm O W, Jain N C and Corroll E J. 1986. *Veterinary Haematology.* 4th edn. Lia and Fediger, Philadelphia.

Sharma R C, Arora A L, Narula M K and Singh R N. 1999. Characteristic of Garole sheep in India *AGRI.* **26**: 57-64.

Siddiqua A, Mammam M A and Hussain M A. 1989. Some biochemical studies in the blood of goats naturally infected with intestinal parasites. *Indian Veterinary Journal* **66**: 502-04.

Singh R N and Bohra S D J. 1996. Garole sheep a profile (Bengal breed of sheep, locally Known as Garole). *Indian Journal of Small Ruminants* **2**: 38-42.

Snedecor G W and Cochran W G. 1994. *Statistical Methods.* 9th edn. Oxford and IBM Publishing Co., Delhi.

Vegad J L. 1995. *Textbook of Veterinary General Pathology.* 1st edn. Vikas Publishing House put. Ltd, New Delhi. pp. 111.

Wooten I D P and Freeman H. 1982. Biuret method for total serum protein. *Microanalysis in Medical Biochemistry.* 6th edn, pp. 144-45 Churchill living stone, London, U.K.

Indian Journal of Animal Sciences 73 (10): 1123-1125, October 2003

Histomorphological and biochemical studies of triclopyrbutotyl ester in goats

T K SAR[1], T K MANDAL[2], S K DAS[3], A K CHAKRABORTY[4], A BHATTACHARYYA[5] and A CHOWDHURY[6]

West Bengal University of Animal and Fishery Sciences, Mohanpur, Nadia, West Bengal 741 252

Received: 10 May 2002; Accepted: 5 June 2003

Key words: Cholinesterase, Goat, Metabolites, Triclopyr butotyl ester

Triclopyr butotyl ester (ACTP ester), a newly introduced herbicide structurally related to picloram, is used to control woody plants and many broad-leafed weeds (*e.g.* mettles, docks, brambles, gorse, broom) in grassland, uncultivated land, industrial areas, coniferous forests, plantation crops and rice fields. Recently the herbicide has been introduced in India. But, literature relating to toxicity and identification of metabolites of ACTP ester is not available in animals particularly in goats. Keeping this in view, the present study was planned to investigate the blood level of 2 metabolites and its effect on plasma cholinesterase activity following a single intravenous administration and histomorphological examination following repeated oral dose administration of ACTP ester in goats.

The experimental study was conducted on clinically healthy Black Bengal female (nulliparous) goats of approximately 1½ - 2 years age weighing from 9.5 – 12 kg. The animals were caged individually in custom's made metabolic cage (stainless steel) throughout the experiment. The temperature of the experimental animal room was maintained at 22°C (± 3°C) and provided artificial lighting facilities, *ad lib.* drinking water and standard feed (Juliet *et al.* 1998). Each animal was fasted overnight before administration of ACTP ester with vehicle.

Clinically healthy Black Bengal goats (6) were divided into 2 groups, each having 3 female (nulliparous) goats. Group 1 goats were considered for determination of blood level of 2 metabolites of triclopyr butotyl ester and their effect on plasma cholinesterase activity after single intravenous administration. Group 2 goats were used for histomorphological study following repeated dose oral administration of ticlopyr butotyl ester.

ACTP ester @ 11.88 mg/kg (Sar *et al.* 2002) dissolving in 1 ml glycerin formal was administered intravenously (i/v) to each experimental goat of group 1 through jugular vein. Blood samples were collected from the jugular vein of each experimental goat into heparinized tubes at different time intervals of 0.08, 0.16, 0.25, 0.33, 0.50, 0.67, 1, 2, 3, 4, 6, 8, 12, 24, 48, 60 and 72 hr post administration in 2 sets of test tubes separately. Analysis of ACTP ester and its metabolites were done as by HPLC as described by Sar *et al.* (2002).

For estimation of cholinesterase enzyme activity, heparinized blood (2 ml) was collected from jugular vein before and at 0.16, 0.33, 0.50, 0.67, 1, 2, 3 and 4 hr pd. Blood samples were centrifuged at 3 000 rpm for 20 min and plasma samples were used for estimation of cholinesterase activity (Augustinson 1957) and expressed as µg of acetylcholine hydrolysed/30 min.

Female goats (3) of group 2 were administered orally with ACTP ester @ 79.22 mg/kg (Sar *et al.* 2002) dissolving in 25 ml carboxymethyl cellulose (CMC) for consecutive 7 days. The animals were slaughtered on day 8 and different tissues like liver, kidney, lung, brain, heart, spleen, adrenal gland and ovary were collected and preserved in 10% formal saline till further utilized. Tissues were dehydrated in ascending grades of ethanol and cleared in cedar wood oil. Paraffin blocks were then prepared and section (3 – 5µ thickness) were cut and stained with routine haematoxylin and eosin (Lillie and Fullmer 1976).

Blood level of two metabolites

Two metabolites of triclopyr butotyl ester, viz. 3, 5, 6 trichloro-2 pyridinyloxyacetic acid (triclopyr acid) and 3, 5, 6 trichloro-2 pyridinol (trichloropyridinol) were determined in blood samples of goat following single dose intravenous administration. The blood concentrations of 2 metabolites along with ACTP ester are presented in Table 1.

The maximum concentration of triclopyr acid (5.85 ± 0.73 µg/ml) was observed at 0.08 hr and persisted in blood till 72 hr post dosing. On the other hand, the maximum concentration of trichloropyridinol (12.29 ± 1.64 µg/ml) was achieved at 72 hr pd. The level of trichloropyridinol in blood ranged between 3.13 ± 0.11 and 12.29 ± 1.64 µg/ml. Further, the concentration of trichloropyridinol at different times were higher compared to triclopyr acid in blood of goat. The concentration of ACTP ester was higher compared to the sum

Present address: [1]Research Scholar, [2]Reader, [3]Research Associate, [4]Formerly Reader, Department of Pharmacology and Toxicology, [5]Reader, [6]Professor, Department of Agricultural Chemicals.

Table 1. Blood level of ACTP ester and its two metabolites (triclopyr acid and trichloropyridinol) (μg/ml) in goats following single dose intravenous administration @ 11.85 mg/kg (mean values of 3 replicates with SE)

Time (hr)	ACTP ester	Triclopyr acid	Trichloropyridinol
0.08	42.64 ± 4.26	5.85 ± 0.73	8.89 ± 0.45
0.16	33.94 ± 3.81	4.60 ± 1.26	5.84 ± 1.49
0.25	26.42 ± 3.32	3.76 ± 0.90	5.63 ± 1.30
0.33	22.88 ± 3.22	1.79 ± 0.17	3.83 ± 0.59
0.50	16.42 ± 2.57	1.24 ± 0.25	5.70 ± 1.43
0.66	12.03 ± 2.13	0.96 ± 0.46	3.13 ± 0.11
1.00	10.87 ± 1.44	1.51 ± 0.63	5.80 ± 1.56
2.00	10.42 ± 1.51	2.04 ± 0.98	6.07 ± 0.70
3.00	9.85 ± 1.46	1.58 ± 0.73	3.50 ± 0.67
4.00	9.22 ± 1.36	3.89 ± 2.01	7.18 ± 2.64
6.00	8.44 ± 1.18	3.03 ± 1.43	8.42 ± 0.54
8.00	7.65 ± 1.04	2.77 ± 0.63	5.83 ± 0.49
12.00	6.39 ± 0.69	2.11 ± 0.74	8.10 ± 0.41
24.00	3.41 ± 0.32	1.62 ± 0.48	6.63 ± 0.10
36.00	1.93 ± 0.14	1.78 ± 0.57	5.04 ± 0.34
48.00	BDL	2.05 ± 0.97	4.39 ± 1.82
60.00	BDL	2.79 ± 1.41	10.88 ± 0.01
72.00	BDL	1.95 ± 0.95	12.29 ± 1.64

BDL, Below detection level.

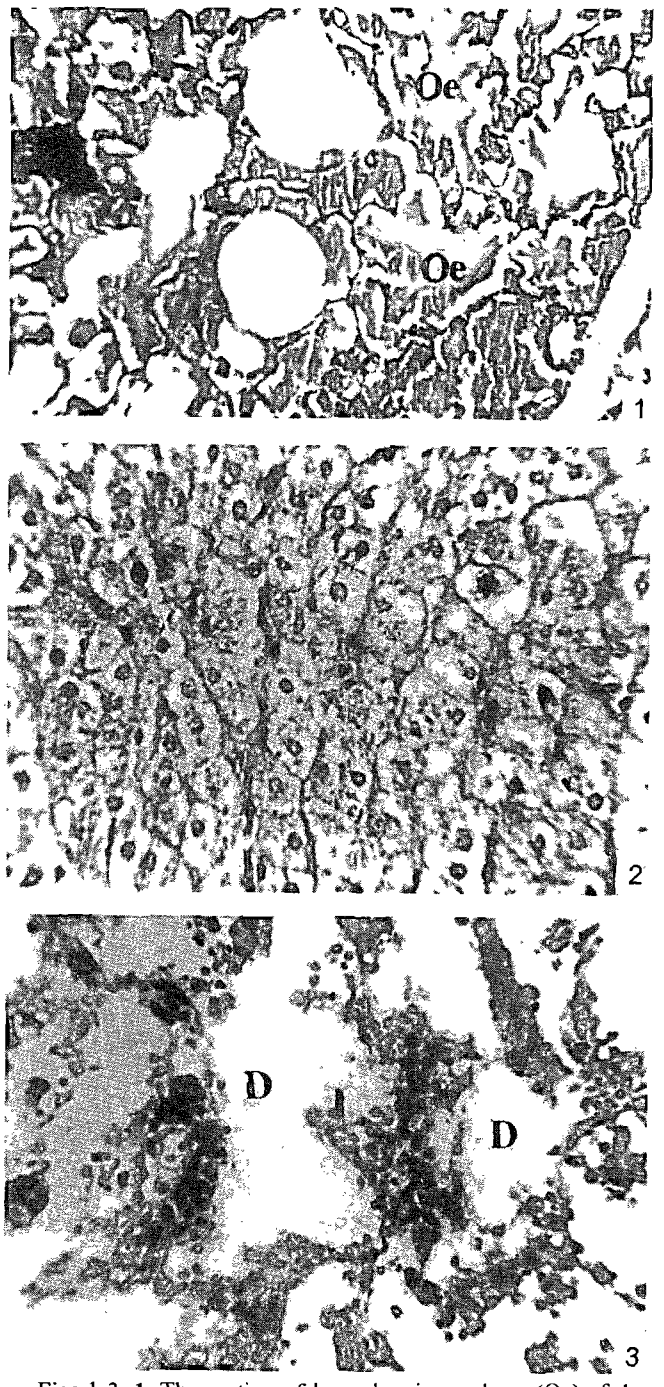
of 2 metabolites till 3 hr pd indicating slow metabolism of ACTP ester in goat.

Plasma cholinesterase activity

Percentage of cholinesterase inhibition was initially lower at 0.16 hr and gradually increased till 1 hr followed by a sharp decline and the enzyme activity returned to basal level at 4 hr of single intravenous administration. ACTP ester achieved its maximum concentration (42.64 ± 4.26 μg/ml) at 0.08 hr followed by a sharp decline up to 1 hr (10.87 ± 1.44 μg/ml) and gradually declined to 1.93 ± 0.14 μg/ml at 36 hr. The concentration of ACTP ester was higher compared to the sum of concentration of its 2 metabolites till 3 hr pd. Triclopyr acid showed its peak level at 0.08 hr and persisted at a lower level up to 72 hr. On the other hand, trichloropyridinol showed its higher concentrations (10.88 ± 0.01 and 12.29 ± 1.64 μg/ml) at 60 and 72 hr pd. Trichloropyridinol maintained a concentration range in between 3.13 ± 0.11 and 8.89 ± 0.45 μg/ml up to 48 hr of single intravenous administration. From this observation, it might be adduced that the 2 metabolites viz., triclopyr acid and trichloropyridinol have no effect on plasma cholinesterase inhibition.

Histomorphological changes

Histomorphological examination of lung showed oedema (Fig. 1). Section of liver revealed disorganization of hepatic cords (Fig. 2). Likewise, the section of spleen showed depletion of lymphocytes in the parenchyma (Fig. 3). But, the section of kidney, brain, heart, adrenal gland and ovary



Figs 1-3. **1.** The section of lung showing oedema (Oe) of the alveoli. H & E., 100 ×. **2.** The section of liver showing disorganization of hepatic cords. H.&E., 450 ×. **3.** The section of spleen showing depletion (D) of lymphocytes in the parenchyma. *H. & E.* 450 ×.

did not present any morphological alteration. Histomorphological examinations revealed that ACTP ester after repeated oral administrations produced mild pathological changes in lung and liver tissue and the lessions are expected to be waned out following discontinuation of therapy. However, ACTP ester induced depletion of lymphocyte in the parenchyma of spleen deserves attention. Spleen is the

store house of T-lymphocytes which is responsible for the production of immunity in the body and this observation demands the necessity of further study.

From this experiment, it may be concluded that ACTP ester undergoes phase I reaction through hydrolysis to form triclopyr acid and trichloropyridinol. Both parent and metabolites have least toxic effect on goats in terms of plasma cholinesterase activity and histomorphology which is important from public health point of view.

SUMMARY

ACTP ester was administered intravenously and blood level of ACTP ester and its metabolites were estimated by HPLC. Histopathology was done after repeated oral administration for 7 days. The concentration of ACTP ester was higher compared to the sum of 2 metabolites indicating slow metabolism of ACTP ester. ACTP ester but not its metabolites inhibited plasma cholinesterase. Histomorphological examination revealed that ACTP ester produced mild pathological changes in lung, liver and spleen tissue.

ACKNOWLEDGEMENTS

We acknowledge the gift of analytical grade ACTP ester and its metabolites by M/s Gharda Chemicals Ltd, Mumbai. Financial assistance was also provided by M/S Gharda Chemical Ltd, Mumbai, India, to carry out the research work.

REFERENCES

Augustinsson K B. 1957. *Assay of Cholinesterase, Methods of Biochemical Analysis.* (Ed.) Glick D. Interscience Publishers Inc.

Juliet S, Mandal T K, Mal B, Chowdhury A, Bhattacharyya A and Chakraborty A K. 1998. Metabolic study of Isoproturon in Goats Following a single oral administration: Toxicokinetics and Recovery. *Journal of Agricultural and Food Chemistry* **46**(1): 178-83.

Lillie R D and Fullmer H M. 1976. *Histopathologic Technique and Practical Histochemistry.* 4th edn. The McGraw – Hill Co., New York.

Sar T K, Bagchi B, Das S K, Mandal T K, Chakraborty A K, Bhattacharyya A and Chowdhury A. 2002. Toxicokinetics, Recovery and Metabolism of triclopyr butotyl (actp) ester in goats. *Journal of Agricultural and Food Chemistry* **50**(15): 4202-209.

Indian Journal of Animal Sciences 73 (10): 1126-1128, October 2003

Ultrasonographic survey of canine gall bladder diseases*

POOJA TIWARI[1], J P VARSHNEY[2] and M HOQUE[3]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received : 20 April 2002; Accepted: 16 June 2003

Key words: Cholecystitis, Dogs, Gall bladder, Hepatobiliary, Sludge, Ultrasonography

Gall bladder disorders in dogs are not as common as in man. Nevertheless, choleliths, cholecystitis, sludge, gall bladder distension (Voros *et al.* 1991, Kirpensteijn *et al.* 1993, Junghee *et al.* 2000, Voros *et al.* 2001) have been reported in dogs. Absence of clinical evidence in canine gall bladder disorders makes the diagnosis difficult. During recent years, biliary ultrasonography has been recommended as a safe and noninvasive technique for confirming the diagnosis of gall bladder disorders (Nyland and Park 1983, Nahrworld 1991, Voros *et al.* 2001). In India, information on canine gall bladder disorders is very scanty (Vijay Kumar *et al.* 2001). The present investigation was therefore undertaken to survey the incidence of gall bladder disorders, if any, in dogs.

Seventy six dogs, with suspected hepatobiliary disorders, brought to referral veterinary polyclinic of the Indian Veterinary Research Institute, were subjected to detailed clinical and sonographic examinations. B-mode grey scale sonography was performed with scanner-200 Vet. (Pie Medical, Netherlands) using 5.0 MHz transducer. Abdominal area was shaved and acoustic gel was liberally applied. The transducer was placed caudal to costal arch with the beam directed dorso-cranially. Imaging was performed in dorsal right and lateral inter-coastal approach using saggital, transverse and dorsal planes. Ultrasonograms were evaluated for size, shape, contours, contents and wall thickness of the gall bladder. Images were recorded on thermal printing paper using black and white thermal printers.

The gall bladder and bile duct were examined in 76 dogs with suspected hepatobiliary disorders. The normal gall bladder, observed in 40 dogs, was anechoic round to oval structure with well defined margins like any other fluid filled strucutre and produced acoustic enhancement in the tissues deep to the gall bladder. Size of the gall bladder was variable probably due to variation in time between feeding and taking ultrasonographs (Nyland *et al.* 1989). The evaluation of gall bladder size was subjective and its dimensions varied according to imaging position and possibily also to its functional state (Voros *et al.* 1991). Distension of gall bladder, sludge, thickening of gall bladder wall, cholelith and biliary obstruction either alone or in combination were observed in 25, 17, 15, 1 and 2 dogs out of 36 dogs. Distension of gall bladder either alone, with sludge and or thick gall bladder wall was the most common finding.

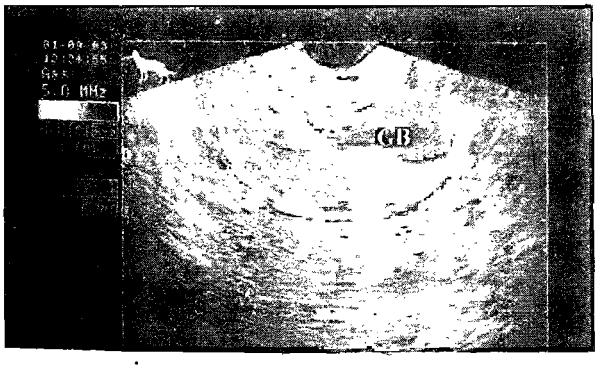
Sludge, a well defined mobile hyperechoic material gravitated to the dependent position of the gall bladder was also seen in 17 dogs. Causes for strands of ecogenic material are nonspecific but more likely to be associated with inflammation (Spaulding 1993). Biliary sludge is known as a precursor of cholelithiasis in humans, but its clinical significance in dogs is not fully understood (Nyland *et al.* 1995, Bromel *et al.* 1998). It could be associated with stasis. However, careful examination of biliary sludge is important owing to its similarity to tumors (Santilli and Biller 1994), mucoceles (Newell *et al.* 1995) and cholecystolithiasis with an acoustic shadowing (Jensen *et al.* 1994). Bromel *et al.* (1998) recorded the prevalence of gall bladder sludge as 53, 62 and 48% among healthy dogs, dogs with hepatobiliary diseases and dogs with other diseases respectively. Gall bladder distension, bile stasis and sludge observed in these cases was in association with either hepatitis, cirrhosis or intraphepatic portosystemic shunt except in 4 cases where gall bladder distension was not associated with any obvious functional or morphological abnormalities of gall bladder or of the liver and appeared to be due to chronic anorexia (Lamb 1991). Cholelithiasis was reocrded in only 1 dog. It seems to be a rare cause of biliary obstruction (Schall *et al.* 1973) and cholecystitis. No clinical signs related to the disease were observed in this case agreeing with the observations of Harris *et al.* (1984). Clinical signs are manifested when cholelithiasis is associated with cholecystitis, biliary obstruction or rupture of biliary tree (Mullowney and Tennant 1982). Hence, clinical significance of cholelithiasis alone is not clear (Nyland *et al.* 1995, Bromel *et al.* 1998). The high occurrence of sludge in gall bladder (17 cases) and only a solitary case of cholelithiasis lend support to the view that biliary sludge rarely results in

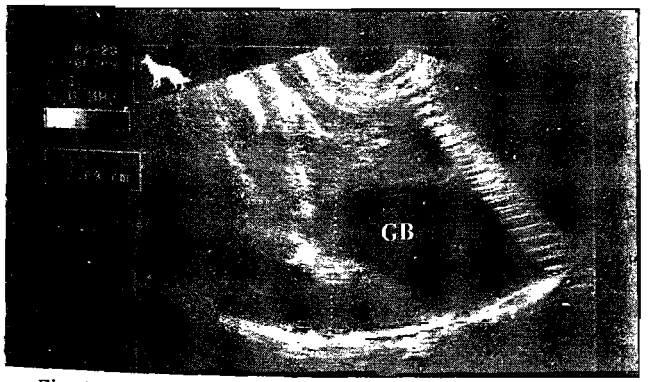
*Part of research work submitted for the award of M.V.Sc. degree by the first author

Present address: [1]C/o Dr Alok Dixit, TDVS, Room No.7, Composite Building, Bilaspur, Chhatisgarh, [2]Prinicpal Scientist, Division of Medicine, [3]Senior Scientist, Division of Surgery

cholelith formation (Kirpensteijn *et al.* 1993).

Gall bladder wall of more than 3.5 mm thickness, observed in 15 dogs, was diagnosed as thickened wall (Sanders 1980, Mittelstaedt 1987) and classed as cholecystitis (Fig. 1). This observation agrees with the recent report of Vijaykumar *et al.* (2001). In B-mode grey scale sonography, thick wall of the gall bladder appeared as hyperechoic region beween 2 echogenic lines. Since thickness was diffused and uniform in most of these dogs, it appeared to be true thickness. Diffused thickening of gall bladder wall was once considered as highly specific for cholecystitis, but now its sensitivity or specificity for an inflammatory process is debated as peritonial effusion also causes pseudothickening of gall bladder wall (Spaulding 1993). In humans, 56 to 75% of the patient with acute cholecystitis and less than 25% of patient with chronic cholecystitis have been reported with diffusely thickened gall bladder wall (Sanders 1980, Rumack *et al.* 1991). Observation of ascites in dogs with thickened gall bladder wall agreed with the observations of Lewandowski and Weinsberg (1981). Cystic mucosal hyperplasia, infectious canine hepalitis, pancreatitis, chronic bile duct obstruction, renal failure and over hydration have been described as possible cause of thickening of the gall bladder wall in dogs (Slatter 1953, Jubb *et al.* 1993). In cholecystitis true thickening of gall bladder wall is attributed to increased vascular permeability and cellular infiltration. The etiology of cholecystitis in dogs is poorly understood, but micro-organisms are considered to play an important role.





Figs 1-2. 1. Ultrasonographic appearence of a canine gall bladder with thickened wall suggestive & cholecystitis. 2. Ultrasonographic appearence of a canine gall bladder with distension and obstructed cranial end.

Biliary obstruction observed in 2 dogs was associated with thickening of gall bladder wall, distension (Fig. 2) and marked icterus (Nyland *et al.* 1995).

ALT (8 to 170 U/ml), SAP (4.0 to 272.88 U/lit), γ GT (3.47 to 219.44 U/lit), total serum protein (3.9 to 8.875 g/dl), serum albumin (1.23 to 4.02 g/dl), blood urea (40.0 to 361.07 mg/dl), serum creatinine (0.90 to 10.06 mg/dl), total bilirubin (0.262 to 27.10 mg/dl) direct bilirubin (0.197 to 24.10 mg/dl) values were highly variable in these cases. Elevated ALT, SAP, GT, total and direct bilirubin levels clearly indicated the involvement of hepatobiliary system. In cases of biliary obstruction mean values of γ-GT (219.44 U/m), SAP (124.06 U/ml), total bilirubin (16.59 mg/dl) direct bilirubin (12.4 mg/dl) and blood urea (158.2 mg/dl) were distinctly higher and total proteins (4.195 g/dl) and serum albumin (2.675 g/dl) were on lower side. The change in liver enzymes, plasma proteins and bilirubin in these cases were in agreement with the observations of other workers (Cosenza 1984, Kirpensteijn *et al.* 1993) in cases of cholangiohepatitis, cholecystitis and cholelithiasis.

SUMMARY

Seventy six dogs with suspected hepatobiliary disease were subjected to B-mode ultrasonography. Of 36 cholecystic disease distension of gall bladder, sludge, thickening (≥3.5 mm) of gall bladder wall, cholelith and biliary obstruction were observed either alone or in combination in 25, 17, 15, 1 and 2 dogs respectively. In other 40 dogs, gall bladder was normal showing anechoic round to oval strucutre with well defined margins. In biliary obstruction values of ALT, SAP, v-GT, total and direct bilirution were distinctly higher and of total serum protein and albumin were lower.

REFERENCES

Bromel C, Barthez P Y, Leveille R and Serivani V. 1998. Prevalence of gall bladder sludge in dogs as assessed by ultrasonography. *Veterinary Radiology and Ultrasound* **39**: 206-10.

Cosenza S F. 1984. Cholelithiasis and choledolithiasis in a dog. *Journal of Veterinary Medical American Association* **184**: 87-88.

Harris S J, Simpson J W and Thoday K L. 1984. Obstructive cholelithiasis and gall bladder rupture in a dog. *Journal of Small Animal Practice* **25**: 661-67.

Jensen A L, Koch J and Snekvik K. 1994. Unusual ultrasonographic presentation of cholelithiasis in a dog. *Journal of Small Animal Practice* **35**: 420-22.

Jubb K V F, Kennedy P C and Palmer N. 1993. *Pathology of Domestic Animals.* 4th edn, pp. 359-66. Academic Press, Inc., San Diego.

Junghee Y, Jinyoung H, Gukjin H, Dongwoo C and Youngwon L. 2000. Cholelithiasis and choledolithiasis in a dog. *Korean Journal of Veterinary Clinical Medicine* **35**: 163-67.

Kirpensteijn J, Fingland R B, Ulrich T, Kikkema D A and Allen S W. 1993. *Choleliths in Dogs*: 29 cases (1980-1990). *Journal of American Veterinary Medical Association* **202**: 1137-42.

Lamb C R. 1990. Abdominal ultrasonography in small animals: Examination of the liver, spleen and pancreas. *Journal of Small Animal Practice* **31**: 5-14.

Lewandowski B and Weinsberg F. 1981. Gall bladder wall thickness distorsion by ascites. *American Journal of Roentgenology* **137**: 519-21.

Mittelstaedt C A. 1987. *Abdominal Ultrasound*. pp. 86-155. Churchill Livingstone New York.

Mulloney P C and Tennant B C. 1982. Choledolithiasis in the dog: a review and a report of a case with rupture of common bile duct. *Journal of Small Animal Practice* 23: 631-38.

Nahrwold D L. 1991. *The Biliary System*. In: *Text book of Surgery*. 14th edn, pp. 1050-63. (Ed.) Sabiston D C Jr. WB Saunders Co., Philadelphia.

Newell S M, Selcer B A and Mahaffey M B. 1995. Gallbladder mucocele causing biliary obstruction in two dogs: ultrasonographic, scintigraphic and pathological findings. *Journal of American Animal Hospital Association* **31**: 467-72.

Nyland T G and Park R D. 1983. Hepatic ultrasonography in the dog. *Veterinary Radiology* **24**: 74-84.

Nyland T G, Hager D A and Herring D S. 1989. Sonography of the liver, gall bladder and spleen. *Seminars in Veterinary Medicine and Surgery (Small Animal)* 4: 13-31.

Nyland T G, Matton J S and Wisner E R. 1995. *Ultrasonography of the Liver*. In: *Veterinary Diagnostic Ultrasound*. pp. 52-73. (Ed) Nyland T G and Matton J S. W B Saunders Co., Philadelphia.

Rumack C M, Wilson S R and Charboneu J W. 1991. *Diagnostic Utrasound*. pp. 106-44. St Lowis, Missouri: Mosby – Year Book Inc.

Sanders R C. 1980. The signficance of sonographic gall bladder wall thickening. *Journal of Clinical Ultrasound* **8**: 143-46.

Santilli R A and Biller D S. 1994. Ultrasonography of liver and biliary tract. *Europian Journal of Companion Animal Practice* **4**: 57-66.

Schall W D, Chapman W L and Finco D R. 1973. Cholelithiasis in dogs. *Journal of American Veterinary Medical Association* **163**: 469-72.

Slatter. 1993. *Text Book of Small Animal Surgery*. 2nd edn, pp. 647-48. W B Saunders Co., Philadelphia.

Spaulding K A. 1993. Gall bladder wall thickness. *Veterinary Radiology and Ultrasound* **34**: 270-72.

Vijaykumar G, Thirunavukkarasu P S and Subramanian M. 2001. Cholecystitis in a dog: ultrasonographic diagnosis. *Indian Veterinary Jounral* **78**: 840-41.

Voros K, Vrabely T, Papp L, Horvath L and Karsai F. 1991. Correlation of ultrasonographic and pathomorphological findings in canine hepatic diseases. *Journal of Small Animal Practice* **32**: 627-34.

Voros K, Nemeth T, Vrabely T, Manczur F, Toth J, Magdus M and Perge E. 2001. Ultrasonography and surgery of canine biliary diseases. *Acta Veterinaria Hungarica* **49**: 141-54.

Indian Journal of Animal Sciences 73 (10): 1129-1130, October 2003

Response of anestrus rural buffaloes (*Bubalus bubalis*) to the intravaginal progesterone implant during summer

C SINGH[1]

Rajendra Agricultural University, Patna, Bihar 800 014 India

Received: 4 October 2001; Accepted: 17 June 2003

Key words: Buffaloes, Progesterone implant

The present experiment was conducted on anestrus buffaloes to bring the acyclic buffaloes in cyclicity during summer (April to June) when ambient temperature was ranging from 36° C to 42° C. Buffalo cows (10, second to third lactation) not coming in estrus from 10 to 15 months after calving and 5 buffalo heifers of 3.5 to 4.5 years of age declared anestrus under small farm holding management system maintained in rural area within the redius of 10 to 15 km of Patna were located. Their genitalia were examined per rectum. None of the buffalo cows and heifers had either corpus luteum or follicle on overy. Progesterone (1.9 g) was implanted intravaginally to all the animals (day 0) and was kept as such for 14 days. Implants were removed on day 14 and animals were examined per recturm once daily for 6 to 7 days after implant removal.

A little swelling of external genitalia with thin vaginal discharge was observed on days 12 to 14 after CIDR implant in 7 buffaloes and 4 heifers. Uterine tone with growing follicles on the ovaries was observed in these buffaloes and heifers on days 2 and 3 of implant removal. Subsequently 5 buffalo cows and 2 buffalo heifers exhibited sign of estrus on day 3 to 5 after implant removal. On natural mating they conceived. The remaining 2 buffaloes and 2 heifer having little uterine tone and developing folicle on days 2 and 3 after implant removal did not exhibit estrus and their follicles became atretic as C L was not detected on their ovaries on days 12 to 14 after implant removal. The rest 3 buffaloes and 1 heifer did not respond to the CIDR implant. The buffalo cows and heifers exhibited the estrus behaviour were allowed for natural mating and conceived.

Absence of palpable corpus luteum on the ovaries of buffaloes indicated that these bufaloes were either true anestrus or some of them might have luteal tissue embeded in ovarian cortex as corpus luteum of buffaloes beyoned 30 days after ovulation is embeded in the overian cortex (Singh and Madan 2000). The response of CIDR implant in 7 out of 10 buffalo cows and 4 out of 5 buffalo heifers in terms of follicular development and presence of uterine tone on days 2 to 3 after implant removal suggested that the circulating level of progesterone might have increased during CIDR implant in these buffaloes. That increased circulatory concentration of progesterone might have sensitiesd the hypothalmic-pituitary system and also at the same time suppressed the GnRH release from the hypothalamus and pituitary level for release, respectively, of GnRH and FSH. (Hafez 1987). Additionally elevation of progesterone before onset of ovarian cyclicity, works as primer sensitizer on the level of hypothalamus and piuitary (Gonzalez Padilla *et al.* 1975a) and for the reception of other reproductive harmones. The exhibition of sign of estrus and conception after natural mating in 5 buffaloes and 2 heifers indicated that these animals responded well and ovulated following progesterone implant. Lack of response to implant in 3 buffalo cows revealed that these buffaloes have either luteal tissue secreting threshold level of progesterone that inhibited hypothalmic pituitary system or these system did not respond to the progesterone released from CIDR. As higher than basal concenteration of progesterone inhibit the release of GnRH and gonodotrophin from hypothalamus and pituitary gland (Hafez 1987). The observation suggests that anestrus buffaloes can be bred by raising the progesterone level through intravaginal progesterone implant even during summer.

Present address: [1]Associate Professor and Head, Department of Veterinary Physiology.

SUMMARY

Progesterone releasing device in form of implant (1.9 g progesterone) was implanted intravaginally for 14 days in 10 noncycling buffalo cows and 5 buffalo heifers maintained in village condition in summer (April to June, ambient temperature 36° to 42° C). Implant was removed on day 14. Response of progesterone implant was observed in both buffalo cows (7) and heifer (4) in terms of vaginal discharge on days 12 to 14 of implant insertion. Buffalo cows (5) and buffalo heifer(2) exhibited overt sign of estrus on days 3 to 5 after implant removal and conceived following natural service.

ACKNOWLEDGEMENT

Author thanks ICAR, New Delhi, India for financial sanction to carry out the investigation under the ad-hoc research project. "Augmentation of reproduction in buffaloes."

REFERENCES

Gonzalez-Padilla E, Niswender G D and Wiltbank J N. 1975. Puberty in beef heifers II Effect of injection of progesterone and estradiol-17 ß on serum LH, FSH and ovarian activity. *Journal of Animal Sciences* **40** : 1105-09.

Gonzalez-Padilla E, Ruiz R, Fever DL, Denham A and Wiltbank J N. 1975 b. Puberty is beef heifers III Induction of fertile estr *Journal of Animal Sciences* **40** : 1100-18.

Hafez ESE. 1987. *Reproduction in Farm Animals*. 5th edn. Lea and Fibigar, Pheladalphia.

Singh C and Madan M L. 2000. Effect of PGF2 α and GnRH during different ovarian status at onset of puberty in Murrah buffalo (*Bubalus bubalis*). *Asian - Australasian Journal of Animal Sciences* **13** : 1059-62.

Indian Journal of Animal Sciences 73 (10): 1131-1132, October 2003

Spontaneous cutaneous neoplasms in buffaloes (*Bubalus bubalis*)

H K BITHU[1], H DADHICH[2] and U K VYAS[3]

Rajasthan Agricultural University, Bikaner, Rajasthan 334 001 India

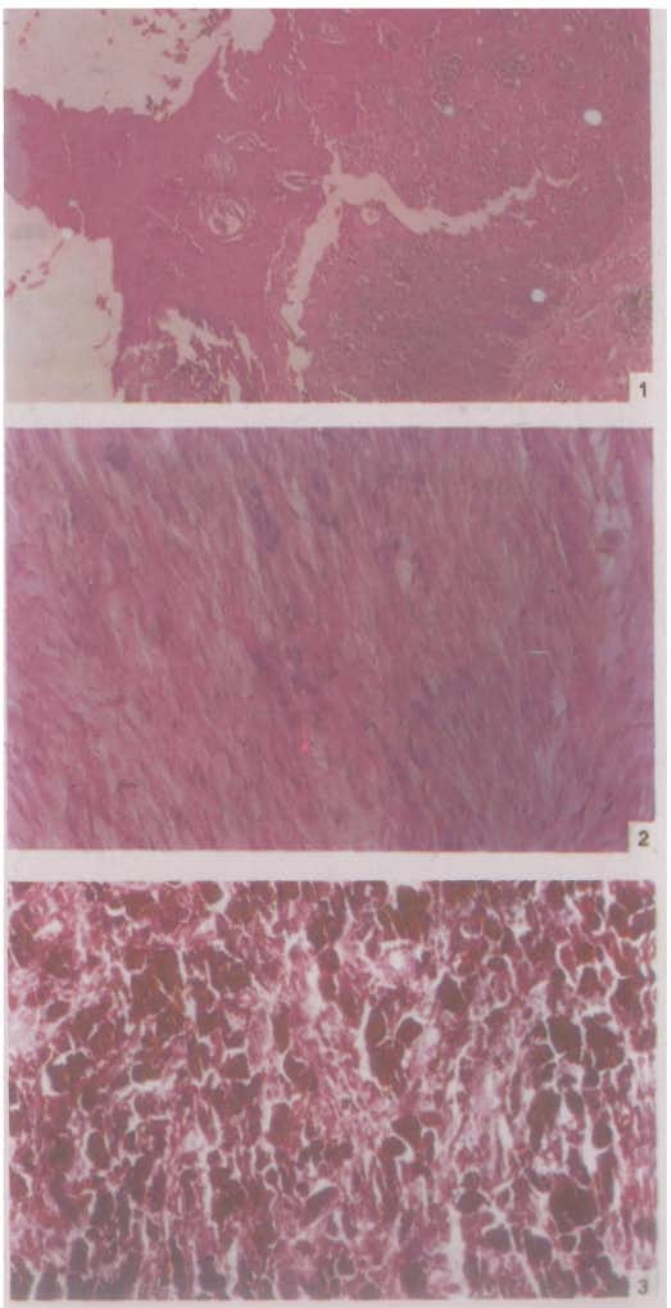
Received: 27 June 2002; Accepted: 5 June 2003

Key words: Buffaloes, Neoplasms

Cutaneous tumors are the most frequently diagnosed neoplastic disorders in domestic animals because they can be identified easily and the constant exposure of the skin to the external environment predisposes this organ to neoplastic transformation. Although information on cutaneous neoplasms in buffaloes is limited except a few reports on melanoma. This communication reports 3 types of cutaneous neoplasms in Indian buffaloes. Buffaloes (2226) were examined at the various slaughterhouses and zoo of Bikaner. Of these, 360 skin samples showing frank lesions were collected in 10% formal saline, processed for paraffin embedding, sectioning (5-6μ) and stained with haematoxylin and eosine for histopathological examination.

In this study, papilloma (1, 1.94%), fibroma (2, 0.56%), melanoma (3, 0.83%) were recorded. Histologically, the papillomas revealed that the epithelium constituting the tumour was very much thickened and grows in finger like projections. A core of connective tissue was present accompanying the growth and the blood vessels were found in the connective tissue (Fig. 1). These changes were similar to those reported in bovine (Cappellaro *et al.* 1980, Jones and Hunt 1983, Bhowmik and Nandi 1986, Lall 1994).

In fibroma there were bundles of spindle shaped fibroblasts and loose collagen fibres (Fig. 2). The tumour cells had large elongated nuclei which confirmate the reports in bovine (Moulton 1961). Melanoma showed heavy intracellular and intercellular deposition of melanin in the epidermis and dermis (Fig. 3). Cellular details were almost obscured by melanin. These changes were similar to those reported in buffaloes



Figs 1-3. **1.** Papilloma showing finger like projections of epidermis along with connective tissue core. H & E, × 100. **2.** Fibroma showing bundles of spindle shaped fibroblasts and loose collagen fibres. H & E, × 100 **3.** Melanoma showing intracellular and intercellular deposition of melanin in the dermis. H & E, × 200

*A part of M.V.Sc. Thesis of first author submitted to Rajasthan Agricultural University, Bikaner.

Present address: [1]JRF, N.R.C. on Camel, Bikaner. [2]Assistant Professor, [3]Formerly Professor and Head, Department of Veterinary Pathology, College of Veterinary and Animal Science.

(Damodaran 1957, Narayan and Rao 1960, Moulton 1961, Paikne *et al.* 1971, Marudwar *et al.* 1978, Ganesh *et al.* 1990, Reddy and Naidu 1994). Further nests of heavily pigmented melanocytes were seen scattered mainly near the dermal-epidermal junction. At places, it appeared that these junctional nests of melanocytes dropped off into the dermis.

SUMMARY

The neoplasms were recorded in 3.33% of the total affected skin samples. Papilloma was found in 1.94% cases revealed that the epithelium constituting the tumour was very much thickened and grow in finger like projections. Fibroma was found in 0.56% cases showing bundles of spindle shaped fibroblasts and loose collagen fibres. Melanoma was found in 0.83% cases showing heavy intracellular and intercellular deposition of melanin in the epidermis and dermis.

ACKNOWLEDGEMENT

The authors thank the Dean, College of Veterinary and Animal Science, Bikaner, for providing the facilities.

REFERENCES

Bhowmik M K and Nandi S N. 1986. The incidence of bovine neoplasms in West Bengal. *Indian Veterinary Journal* **63**: 271-74.

Cappellaro C E M P D M, Ribeiro L O O, Muller S B K and Piegas N S. 1980. Morphology and histopathology of various types of papilloma in bovine. *Biologico* 44: 307-16. (*fide Veterinary Bulletin* **50**: 4238.)

Damodaran S. 1957. Melanomata in buffaloes. *Indian Veterinary Journal* **34**: 325-29.

Ganesh T N, Manickarajan A, Ameerjan K, Balasubramaniam N N and Sundararaj A. 1990. Melanoma and malignant melanoma in bovine-two cases reports. *Indian Veterinary Journal* **67**: 558.

Jones T C and Hunt R D. 1983. *Veterinary Pathology.* 5th edn. (Indian edn.) K.M. Varghese Co., Bombay.

Lall H K. 1994. Prevalence of tumours in domestic animals in Jimma (Ethiopia) with a case record of adrenal tumour in a bullock. *Indian Veterinary Journal* **71**: 80-82.

Marudwar S S, Bhagwat S S and Narkhede M D. 1978. Melanomas in bullocks. *Indian Veterinary Journal* **55**: 667-68.

Moulton J E. 1961. *Tumours in Domestic Animals.* University of California press, Los Angeles.

Narayan J V and Rao S P. 1960. A case of cutaneous malignant melanoma in a she buffalo. *Indian Veterinary Journal* **37**: 468-71.

Paikne D L, Kandhana D L, Deshmukh M S and Kannadkar V S. 1971. Cutaneous melanoma in a calf. *Indian Veterinary Journal* **48**: 631-32.

Reddy K P and Naidu M S. 1994. Malignant melanoma in a bullock. *Indian Veterinary Journal* **71**: 1130-31.

Indian Journal of Animal Sciences 73 (10): 1133-1135, October 2003

Histopathological alterations in the uterus of buffaloes affected with uterine torsion

S PRABHAKAR[1], S S MATHARU[2] and R S BRAR[3]

Punjab Agricultural University, Ludhiana, Punjab141 004 India

Received: 21 February 2002; Accepted : 10 June 2003

Key words: Buffalo, Histopathology, Torsion of uterus, Uterus

Torsion of uterus occurs near term or at the beginning of first or second stage of parturition (Pearson 1971). Strangulation of uterine vessels in uterine torsion cases may lead to arrested blood supply to uterus causing irreparable damage to the uterine tissues. Very little work has been done on the histopathological changes in the uterus of buffaloes affected with uterine torsion. The present study was conducted to investigate histopathological changes induced in uterine tissues as a consequence of uterine torsion.

Buffaloes (25) affected with uterine torsion, presented for treatment at the PAU veterinary clinics were utilized. The buffaloes following complete anamnesis were subjected to either detorsion of uterus by Sharma's modified method (Singh and Nanda 1996) or caesarean section. Buffaloes (15) that were detorted and delivered per vaginum were divided into 2 subgroups, group 1a (<24 hr, n=8) and group 1b (> 24 hr, n= 7), depending on the duration of dystocia.

Buffaloes (10) having dystocia beyond 72 hr (group 2) were delivered through caesarean section and uterine tissues collected. Five normally calving buffaloes were also sampled, which served as control for comparisons.

Uterine tissue samples were collected from both control and dystocia groups immediately after parturition or detorsion where cervix was dilated while in group 2 buffaloes uterine biopsy was collected at the time of caesarean section with the help of a uterine biopsy punch. The tissue samples were preserved in 10% neutral buffered formalin and processed for histopathological studies. Sections (5-6 µm) thick were stained with hematoxylin and eosin (Luna 1968) and evaluated for pathological changes.

Microscopic evaluation of uterine tissues in normally calving buffaloes revealed intact columnar epithelium with focal loss of epithelial cells at certain places (Fig. 1). Few epithelial cells were hypertrophied and had vacuolated appearance. Endometrial glands had small amount of secretory material in their lumen indicating normal activity. Histopathological picture of normal gravid uterus (Bhavsar *et al.* 1974) and uterus during luteal phase (Jones and Hunt 1983) also showed similar picture.

Uterus of buffaloes affected with uterine torsion revealed various degenerative and necrotic changes depending upon duration of dystocia. In most animals of group 1a (< 24 hr), there was patchy loss of endometrial epithelium. The changes in these animals were comparable to normally calving buffaloes as uterine tissues still had normal or nearly normal architecture.

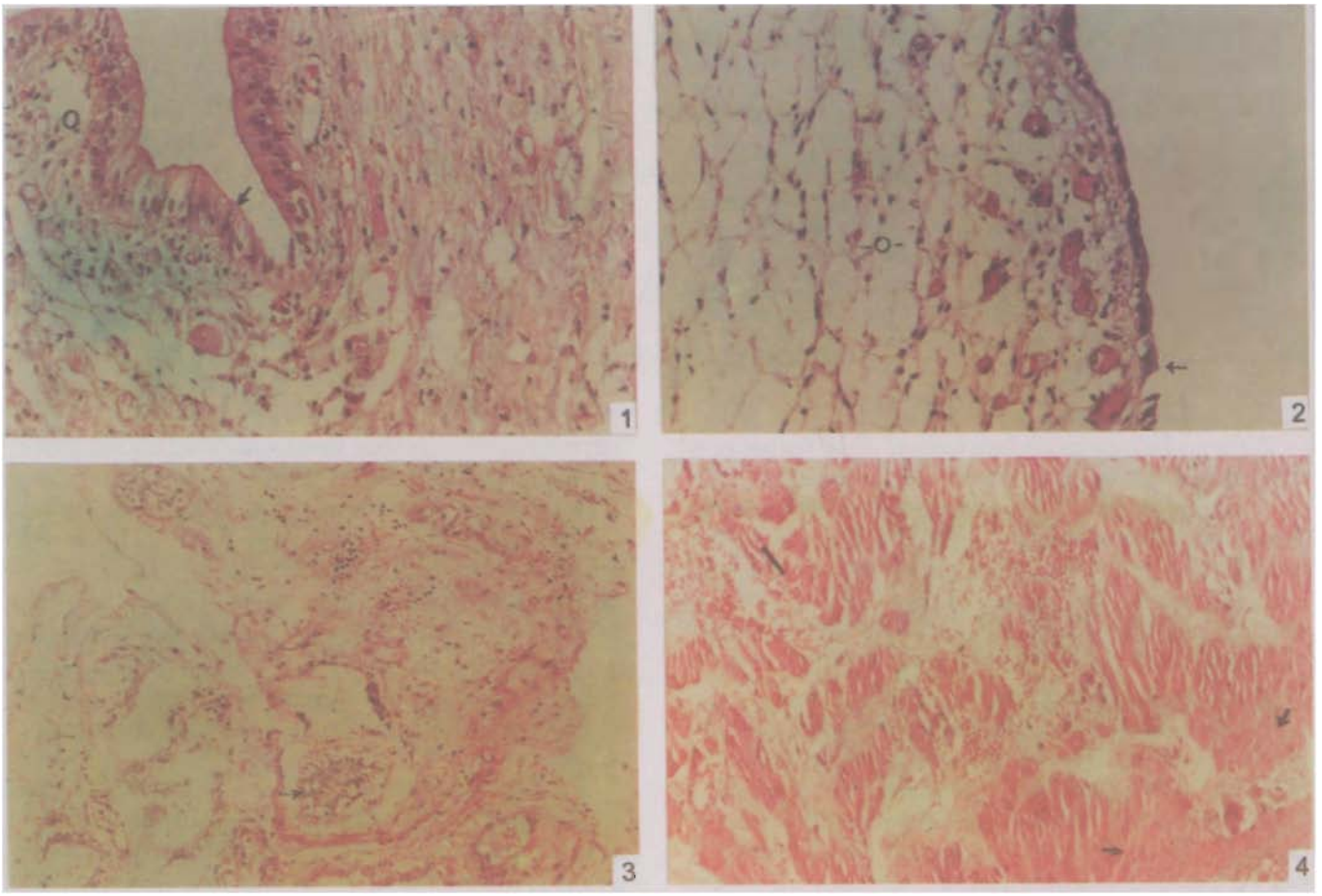
In group 1b, endometrial lining epithelium and basement membrane were mostly absent. The congestion alongwith sub-endometrial oedema and hemorrhages were also sever in cases presented within 24 hr (group1a) (Fig. 2) as compared to that seen in groups 1b and 2 buffaloes, which was perhaps because of circulatory blockade for comparatively shorter period of time. Also, the degenerative and necrotic changes in endometrial glands and myometrium as a whole were more extensive in groups 1b and 2 animals. Among these 2 groups, endometrial glands were not easily recognizable and were in advanced stages of degeneration (Fig. 3).

The lumen of endometrial glands in group 1b buffaloes (>24 hr) was filled with cellular exudates, mostly the RBCs, whereas the endometrial glands in group 1a showed more or less normal glandular integrity. The findings of the present study corroborated the observations of Malik (1986) and Agrawal (1987) who were of the opinion that circulatory arrest leading to anoxia might be responsible for such changes in the uterine tissues.

Uterine muscles appeared stretched, separated and vacuolated due to edema in group 1a buffaloes whereas in group 1b and 2 buffaloes, the myometrium was showing coagulative necrosis alongwith congestion and hemorrhages (Fig. 4). Infiltration of inflammatory cells, predominantly the neutrophils, in myometrium was observed in group 1b animals while in group 2, gangrenous metritis had set in owing to infiltration of saprophytic bacteria which was contrary to the findings of Malik (1986) and Agrawal (1987).

Repeat biopsy samples in group 1b animals at 96 hr showed

Present address: [1]Associate Professor, Department of Animal Reproduction, Gynaecology and Obstetrics. [2]Veterinary Officer, Department of Animal Husbandry and Fisheries, Government of Punjab. [3]Professor, Department of Veterinary Pathology.



Figs 1-4. **1.** Endometrial epithelial lining (arrow) alongwith slight oedema (O) in normally calving buffaloes (control group) immediately after calving (H & E × 180). **2.** Disrupted endometrial epithelial lining (arrow) with extensive sub-endometrial oedema (O) and congestion in torsion affected buffaloes treated within 24 hr (group 1a) (H & E × 180). **3.** Degenerative changes in endometrial glands in buffaloes subjected to caesarean section (group 2) (H & E ×180). **4.** Coagulative necrosis in uterus of torsion affected buffaloes treated after 24 hr (group 1b) (H & E ×180).

that although tissue regeneration and repair had initiated, yet changes in many sections were still marked. It thus appears from the present findings that the extent of damage to the uterine tissue due to uterine torsion varies with the duration of dystocia. In comparatively fresh cases the tissue may undergo regeneration if detorsion is achieved, whereas in sufficiently delayed cases (beyond 72 hr) the uterus undergoes gangrenous and suppurative metritis leading to poor recovery rate.

SUMMARY

In normally calving buffaloes, there was patchy loss of endometrial epithelium with normal endometrial glands and stroma. Histopathological alterations in uterus of uterine torsion affected buffaloes detorted within 24 hr (group 1a), revealed similar picture, whereas in buffaloes detorted after 24 hr (group 1b), the endometrial epithelium and basement membrane were absent and endometrial glands were in advanced stage of degeneration. Repeat biopsy samples in group 1b buffaloes at 96 hr post-detorsion showed tissue regeneration, but the changes were still marked. In buffaloes having uterine torsion for more than 72 hr (group 2) and delivered through caesarean section, the uterine tissue was necrosed, thus, indicating a marked influence of duration of dystocia due to uterine torsion on tissue histomorphology and a poor recovery rate.

REFERENCES

Agrawal R G. 1987. 'Some studies on uterine torsion with special reference to its etiology and treatment in buffaloes'. Ph.D. Dissertation, Punjab Agricultural University, Ludhiana, India.

Bhavsar B K, Pattabhiraman S R and Venkatswami V. 1974. Endometrial changes during early pregnancy of *Bubalus bubalis*. *Indian Veterinary Journal* **51**: 175.

Jones T C and Hunt R D. 1983. *Veterinary Pathology*. 5th edn. Lea and Febiger, Philadelphia.

Luna L G. 1968. *Manual of Histological Staining Methods*. 3rd edn. Armed Forces Institute of Pathology. McGraw-Hill Book Co., New York.

Malik J S. 1986. 'Histochemical and Histomorphological studies on uterine torsion in buffaloes (*Bubalus bubalis*)'. M.V.Sc. Thesis.

Haryana Agricultural University, Hisar, India.

Pearson H. 1971. Uterine torsion in cattle, a review of 168 cases. *Veterinary Record* **89**: 597.

Singh P and Nanda A S. 1996. Treatment of uterine torsion in buffaloes: Modification of Schaffer's method. *Indian Journal of Animal Reproduction* **17**: 33.

Indian Journal of Animal Sciences **73** (10): 1136-1140, October 2003

Somatic cell hybridization betweeen buffalo leucocyte and mouse cell line*

P KUMARASAMY[1], P THANGARAJU[2], P KANAKARAJ[3], V THIAGARAJAN[4] and K KUMANAN[5]

Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 007 India

Received: 10 October 2002; Accepted: 29 May 2003

ABSTRACT

Gene mapping studies in animals will help to localize the genes on their chromosomes. Among various approaches for gene mapping, somatic cell hybridization is comparatively simpler and easily adoptable approach. Somatic cell hybridization between buffalo leucocytes and mouse cell line, LM(TK$^-$) was achieved by suspension fusion technique using polyethylene glycol as fusogen. Effect of duration of PEG exposure, concentration and grade of PEG and parent cell density were evaluated to maximize the hybridization frequency. Hybrid cell colony output was also influenced by density of PEG-treated cells during fusion. The optimum cell ratio was found to be 1: 3 (mouse cell line LM(TK$^-$) and buffalo leucocytes). Buffalo-mouse hybrids were selected in HAT medium and subsequently maintained in growth medium. Both parental cells as well as hybrids colonies were analyzed karyotypically to confirm hybridization between buffalo leucocytes and mouse cell line.

Key words: Somatic cell hybridiation, Buffalo, Mouse cell line

Somatic cell hybridization combined with molecular genetics has served as the most important technique for physical mapping of genes. Extensive studies have been made in human and mouse. In cattle also considerable work has been done in developed countries. However, not much work on buffalo gene mapping has been done, even though buffalo is considered to be the backbone of Dairy Industry in India. Hence, the study was undertaken to standardize somatic cell hybridization.

Mouse and hamster cell lines have been used, as fusion partner to somatic cells from various livestock species (Saidi-Mehtar *et al.* 1981, Womack and Moll 1986). Research on buffalo gene mapping using this technique is of recent origin and only few reports are available (El-Nahas *et al.* 1993, Joshi *et al.* 1995 and Rank *et al.* 1998). The present study aims at standardization of methodology for the production of hybrid cells between river buffalo leucocytes and LM(TK$^-$) (mouse connective tissue) cell-line.

MATERIALS AND METHODS

The 10ml of blood samples were collected from the adult healthy buffaloes brought to artificial insemination ward, Department of Clinics, Madras Veterinary College, Chennai, and allowed to stand for 30 min. Centrifuged at 700-800 rpm for 10 min and discarded supernatant solution. Cell sediments slowly suspended in 5ml of RBC lysis buffer and allowed to stand for 20min at room temperature. Leucocytes, after centrifugation were washed twice in 5 ml PBS or culture medium and finally suspended in 10ml culture medium.

Cell line and strain

The LM (TK$^-$) (mouse connective tissue) cell-line, obtained from National Centre for Cell Sciences (NCCS), Pune, was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and incubated under 5% CO_2 tension at 37°C. This cell line was resistant to 2.6×10^{-8} M azaguanine.

Cell fusion

The suspension fusion technique developed by Davidson and Gerald (1976) was followed with slight modification. Freshly separated leucocytes and LM(TK$^-$) cells at log phase of growth were washed twice with DMEM and mixed and suspended in desired ratio in DMEM. The parent cells were pelleted in round bottom test-tube (Nunc), the medium completely removed and the cells suspended in 1 ml polyethylene glycol (PEG) solution prewarmed at 37°C. PEG solution was poured drop by drop and the cells were mixed gently by continuous agitation. After 30/90/120 sec of exposure, cells were diluted rapidly in 10ml DMEM and washed twice. The cells were again washed once with growth

*Part of Ph.D. Thesis of first author.

Present address: [1]DBT-Postdoctoral Fellow, CCMB, Hyderabad 500007. [2]Dean, [3]Professor, Department of Animal Genetics and Breeding, [4] Professor, [5]Associate Professor, Department of Animal Biotechnology, Madras Veterinary College, Chennai 600 007.

medium and plated in 96- well microtitre plates (Nunc).

Selection of hybrid cells

PEG treated cells were plated by multi-channel pipette in 96-well microtitre plate @ 200ml cell suspension per well. The plates were sealed with adhesive tape after swabbing all sides by denatured spirit. Wells were renewed with fresh HAT selective medium containing hypoxanthine (2.5×10^{-4}M), thymidine (1.6×10^{-5}M) and aminopterin (1.0×10^{-7}M) supplemented with 10 % FBS on every fourth day. Growth of hybrid cells in terms of expanding colonies was recorded by screening the wells under inverted microscope. The wells carrying single colony were trypsinized by using trypsin-versene-glucose (TVG) containing 1% trypsin on confluence and the cells transferred to 24-well plates. The wells were inoculated with 1ml of fresh HAT medium supplemented with 10% FBS. The wells, which grew confluent, were transferred to 24 well plates. The hybrid cells in 24-well plates were refed every third day and on confluence, these were trypsinized and transferred to 6-well plate. Finally these were expanded to TC flasks. Hybrid cell colonies from each well of 96-well microtitre plate were expanded independently.

Maintenance of hybrid cell colonies

The hybrid cell colonies after selection in HAT medium were maintained on growth medium at 37°C under 5% CO_2 tension. On monolayer formation, usually 4 to 5 days after seeding, the clones were subcultured in 1: 4 split ratio and refed every third day after each passaging. Utmost care was taken to avoid any cross contamination among clones and cell lines.

Chromosome preparation

Buffalo leucocyte cultures were harvested for chromosome preparation using standard technique. LM(TK$^-$) and hybrid cells were harvested in log phase of growth, usually 36-48 hr after subculturing. Colchicine was added at the rate of 0.01-0.02 µg/ml for 1 to 3 hr. Medium from TC flask was discarded and cell monolayer was washed with 2 ml fresh medium and the cells were trypsinized. Cells were exposed to hypotonic solution for 15-20 min and given 3 washes with fixative in the same way as for leucocytes. The cell suspension was dropped onto the pre-chilled glass slides; the slides were then air-dried.

RESULTS AND DISCUSSION

Hybridization between buffalo leucocytes and LM(TK$^-$) cells was successfully achieved by suspension fusion technique as reported by Joshi (1995) and Rank (1996) with buffalo fibroblast/ leucocytes. Mouse and hamster cell lines deficient in HPRT or TK have been used to develop somatic cell hybrids by several researchers (Bruns *et al.* 1978, Sparkes *et al.* 1978). Here, attempts were made to maximize the yield of hybrid cell colonies by optimizing various parameters involved in the hybridization of cells.

Effect of concentration and grade of PEG on cell fusion

Fusion between LM(TK$^-$) and buffalo leucocyte at 1: 3 ratio were exposed to PEG for 30, 90 and 120 sec, out of three grades, PEG-1450, PEG-3350 and PEG-4000 at 33, 45 (45% PEG /10% DMSO), 50% concentration were evaluated. The hybridization frequency was scored in terms of number of wells showing hybrid cell colonies on day 16 per 96 well microtitre plates. The results are presented in Table 1. The overall mean values for PEG grade 6.533 ± 1.38, 3.800 ± 0.62 and 2.333 ± 0.56 for PEG-1450, PEG-3350 and PEG-4000 respectively. The overall mean values for PEG concentration were 2.467±0.50, 7.333±1.10 and 2.867±0.87 for 33, 45 (45% PEG/ 10% DMSO), 50% concentration respectively. The PEG 1450 at 45% gave maximum hybridization frequency. However, other concentration gave moderate results as reported by Rank (1996). 50% is widely recognized as an optimum concentration of PEG for somatic cell hybridization. Any concentration higher than this resulted into marked cell damage while lower than this gives suboptimum hybridization frequency (Davidson and Gerald 1976, Gefter *et al.* 1977, Goding 1983). However, certain cell types are sensitive to this concentration and do not yield acceptable level of hybridization (Davidson and Gerald 1976). DMSO was incorporated at 45% concentration at 10% level, as it reduced toxicity and widened the range over which PEG is effective (Norwood *et al.* 1976).

Reduction in PEG concentration with the inclusion of DMSO resulted in an improvement in hybridization frequency. However, reduction in PEG concentration did not have equal quantitative effect at different grades and it was evident that there was a marginal decline in hybrid cell colony when PEG-1450 was used as fusogen. High molecular grade PEG (MW-4000) at 50% concentration was found to be toxic to the cells; hence dilution of PEG concentration along with protective coverage conferred by DMSO might result into improvement of hybridization frequency. But lower molecular grade PEG (MW 1450) at same concentration might not be

Table 1. Effect of PEG concentration and grade on hybridization frequency in suspension fusion technique

PEG concentration/ grade	Number of wells with hybrid cell colonies day 16 /96 wells			
	PEG 33%	45%PEG+ 10%DMSO	PEG 50%	Overall
PEG 1450	12	62	24	$6.533^{b} \pm 1.38$
PEG-3350	18	28	11	$3.800^{a} \pm 0.62$
PEG-4000	7	20	8	$2.333^{a} \pm 0.56$
Overall	2.467^{x} ±0.50	7.333^{y} ±1.10	2.867^{x} ± 0.87	-

Means bearing different superscripts between rows (a,b) and between columns (x,y) differ significantly (P<0.01).

toxic and hence, lower concentration of PEG could not have improved the hybridization frequency. Beneficial effect of dilution of PEG concentration to 45% and incorporation of DMSO was also reported by Rank *et al.* (1998) in buffalo - mouse somatic cell hybridization. Use of PEG in wide range of grades for successful hybridization of variety of cells had been described by Ryttman *et al.* (1986), Hallerman *et al.* (1988) and Joshi *et al.* (1995).

Among the six concentrations - grade combinations, PEG-1450 at 45% concentration gave the best (6.533 ± 1.38, 7.333 ± 1.10 respectively) results. Davidson and Gerald (1976) observed peak hybridization at 50% concentration of PEG-1000 among the range of concentrations (40 to 70 %) and grades (MW 200 to 6000) tested with buffalo leucocytes and rodent cells (RAG,Wg3h,Yh21 and E36).

Effect of duration of PEG exposure

Effect of duration of PEG exposure was evaluated in terms of number of wells showing hybrid cell colonies and total number of colonies in 96-well microtitre plate depicted in Table 2. The overall mean values were 7.667 ± 1.29, 57.667 ± 3.92 and 15.000 ± 2.22 for 30, 90 and 120 seconds respectively. PEG-1450 at 45% (45% PEG and 10% DMSO) exposed for 90 sec on LM(TK$^-$) and buffalo leucocytes gave maximum hybridization frequency as compared to 30 and 120 sec reported by Rank (1996) with RAG and buffalo leucocytes. Although there are reports on successful hybridization with prolonged exposure to PEG for 5 to 15 min (Pontecorvo 1975), majority of gene mapping studies reported that the duration of PEG exposure for 2 or less than 2 min. In the present study too, parent cells were exposed to PEG for 30 to 120 sec to identify an optimum time of PEG exposure for fusion of leucocytes by suspension fusion technique. When the cells were exposed for 30 sec, the frequency of hybridization was unsatisfactory (7.667±1.27). When the duration of PEG exposure was prolonged, hybridization frequency also increased and the maximum hybridization frequency (57.667±3.92) was achieved at 90 sec. Any further exposure diminished the hybridization frequency.

Table 2. Effect of duration of PEG exposure to parental cells on hybridization frequency in suspension fusion technique

Exposure time	Number of wells showing hybrid cell colonies/96 wells			
	Day 7	Day 16	Day 24	Overall
30 sec	2	8	13	7.667^a ± 1.27
90 sec	39	66	68	57.667^c ± 3.92
120 sec	9	14	22	15.000^b ± 2.22
Overall	16.667^x ± 4.55	29.333^y ± 7.09	34.333^y ± 6.57	

Means bearing different superscripts between rows (a,b,c) and between columns (x,y) differ significantly (P<0.01)

Work on hybridoma (Goding 1983) revealed that the rate of fusion and toxicity to the cell increases with increased duration of PEG exposure. In the present study, it appears that increased output of hybrid cell colonies was due to the improvement in rate of fusion but the drop in hybrid cell output at 120 sec was most logically due to reduced cell viability on account of PEG approaching toxic level. Gefter *et al.* (1977) also observed that parent cells could tolerate prolonged exposure (of up to 7 min) to PEG at lower concentration (35-40%) however toxicity was evident in most circumstances, beyond 2 min of exposure to PEG at 50% concentration. Davidson and Gerald (1976) have shown that PEG exposure for 60 sec in suspension fusion of leucocytes and mouse cell line gave hybridization frequency (7.8 colonies) better than that obtained at PEG exposure for 30 or 120 sec (3.6 and 1.0 colonies) with RAG cells. However, they did not evaluate the effect of PEG exposure for 90 sec. In this context, it is pertinent to mention that there are abundant reports on gene mapping describing somatic cell hybridization effected by PEG exposure for 30 or 120 sec. However, in majority of experiments the hybrids were derived either by monolayer or by pancake technique and only a few reports described suspension fusion.

In the present study, it was found that PEG exposure for 90 sec was optimal (57.667 ± 3.92%) for suspension fusion of leucocytes. The finding seems justifiable with the earlier observation that the cells, which fused well at a particular exposure in one technique, required a different exposure time in another technique (O'Malley and Davidson 1977).

Effect of parent cell density

Density dependent growth of hybridomas is well documented in literature. Hence, to explore possible effect of cell density on growth of buffalo-mouse hybrids, parental cells (LM(TK$^-$) and buffalo leucocytes) in 1: 1 {(2.5: 2.5)x10^6}, 1: 3 {(2.5: 7.5)x10^6}, and 1: 5 {(2.5: 12.5)x10^6} proportions were treated with PEG-1450 at 45% (45% PEG±10% DMSO) concentration for 90 sec and these PEG-treated cells were cultured in 96-well microtitre plates. Hybridization frequency was assessed in terms of number of wells carrying hybrid cell colonies on day-16 (Table 3, The overall mean values were 2.80 ± 0.86, 26.60 ± 5.33 and 17.20 ± 3.34 in parental cells of 1: 1, 1: 3 and 1: 5 proportions respectively. Hybrid cell colonies were observed in maximum number of wells when the cells fused at 1: 3 (LM(TK$^-$) and buffalo leucocytes) ratio. Other proportions gave moderate hybrid cell colonies.

In the present experiment, growth of hybrid cell colonies was also found to be highly density-dependent. Maximum hybrid cell colonies could be obtained at 1: 3 ratio (2.5 × 10^6 and 7.5 × 10^6 cells of LMTK and buffalo leucocytes) and seeded at 1 × 10^5 cells/well. Cell density lower or higher than this, resulted in a decreased hybridization frequency. Wells, which received cell population higher than 1 × 10^5 cells/well,

Table 3. Effect of cell density on growth of hybrid cell colonies in suspension fusion technique

Cell density LM (TK^-)±Buffalo leucocytes	Number of wells showing hybrid cell colonies at day-10/96 wells by five trials	Total number of colonies	Overall
1: 1 (2.5: 2.5) × 10^6	0, 2, 3, 5, 4	14	$2.80^a \pm 0.86$
1 3 (2.5: 7.5) × 10^6	17, 28, 14, 30, 44	133	$26.60^b \pm 5.33$
1: 5 (2.5: 12.5) × 10^6	12, 26, 23, 17, 8	84	$17.20^{ab} \pm 3.34$

Means bearing different superscripts differ significantly ($P<0.01$).

are also expected to receive proportionally more hybrid cells. Despite more hybrid cells in these wells, number of hybrid cell colonies obtained was lesser than those wells, which received 1 × 10^5 cells. This retractile growth of hyorid cell colonies at higher cell concentration might be due to competence among cells for nutrients and substrate. On the other hand, very less hybrid cell colonies at 1: 5 ratio of fusion and seeding more than 1 × 10^5 cells/well seeding rate, could be attributed to less number of hybrid cells. Density depending nature of hybridomas was also observed by Olsson and Kaplan (1983). They found maximum yield of viable hybrids at 2 × 10^5 cells/well, drastically low at 5 × 10^5 cells/well while, absolutely no colonies observed beyond this density.

The density dependent nature of hybrid cells is important while cloning by limiting dilution method. Feeder cells are generally used to support the growth of newly formed hybridomas. In the present study, cloning was not done, instead the PEG treated parent cells were placed in HAT medium in which they could not grow, but remain alive atleast for few days to serve as feeder cells to newly formed highly fragile hybrids.

It is noteworthy that the studies, which demonstrated the effect of cell concentration, refer to concentration of parent cells before fusion (Davidson *et al.* 1976, O'Malley and Davidson 1977, Joshi *et al.* 1995). These studies have shown that cell-to-cell contact during PEG treatment was an important factor in deciding the frequency of hybridization.

Karyotypic characterization of parental and hybrid cells

The modal chromosomal number in buffalo 2n = 50. GTG-banded metaphase chromosomes of buffalo consisted of 5 pairs of submetacentric and 20 pairs of acrocentric chromosomes including a pair of acrocentric sex chromosomes. X-chromosome was the largest acrocentric, while Y-chromosome was similar in size to that of chromosome 22. All chromosomes present G-negative centromeric region. GTG-banded metaphase chromosomes of buffalo were identified as per the standard karyotype generated by Iannuzzi (1994).

Sixty metaphase chromosomal spreads of LM(TK^-) cells were analyzed and the modal chromosome number determined as 44 presented in Table 4. Biarmed chromosome ranged from 4 to 11. The metaphase chromosomes, when GTG banded showed G-positive centromeric region. Chen (1987) also has investigated the karyotypic characteristics of LM (TK) and reported chromosome variation in narrow range (41 to 47) with modal number 44/45.

In LM(TK^-) and buffalo hybrid cell, the modal chromosome complement comprised 84 chromosomes presented in Table 4 with 10 to 21 biarmed chromosomes.

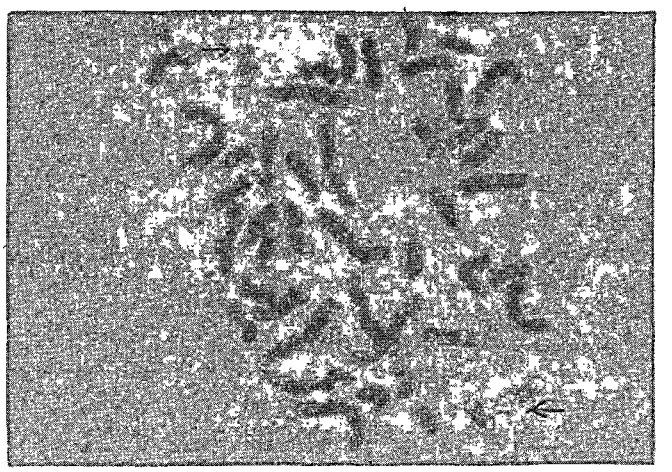


Fig 1. Partial mitotic metaphase chromormal spread of buffalo-LM (T K) hybrid cells (arrow indicating the Forth chromonome of buffalo)

Table 4. Distribution of chromosome numbers in LM(TK^-), buffalo and hybrids {LM(TK^-) and buffalo}

Cell type	Mean	S.D	Per cent cells with modal chromosome number	Number of chromosomes/number of cells
LM(TK^-)	44	1.66	36.6	41, 42, 43, 44, 46, 47
Buffalo	50	0	100.0	3, 3, 13, 22, 11, 8, 50 40
Hybrids {LM(TK^-) + Buffalo}	84	3.47	57.8	76, 77, 80, 82, 84, 89, 92, 96 1, 2, 3, 12, 37, 6, 1, 2

On GTG banding, it was observed that the mouse chromosomes showed dark centromeric regions while the buffalo chromosomes had faint centromeric region (Fig.1). Di Berardino and Iannuzzi (1982) had also reported similar observations.

ACKNOWLEDGEMENT

The part of the thesis submitted by the first author to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, approved the thesis for the post-graduate degree is highly acknowledged. We thank NCCS, Pune, for providing LM(TK$^-$) cell line. CSIR, New Delhi, is acknowledged for granting of Senior Research Fellowship to the first author for this work.

REFERENCES

Bruns G A P, Leary A C, Eisenman R E, Bazinet C W, Regina V M and Gerald P S. 1978. Expression of ACONs and GALT in man-rodent somatic cell hybrids. *Cytogenetics and Cell Genetics* **22**: 172-76.

Chen T R. 1987. Cytogenetics of somatic cell hybrids. 2. Modal karyotypes and mosaicism in mouse mutant cell lines: LM(TK$^-$), A9, RAG. *Cancer Genetics and Cytogenetics* **24**: 95-107.

Davidson R L and Gerald P S. 1976. Improved techniques for the induction of mammalian cell hybridization by polyethylene glycol. *Somatic Cell Genetics* **2**: 165-76.

Davidson R L, O'Malley K A and Wheeler T B. 1976. Polyethylene glycol induced mammalian cell hybridization: Effect of polyethylene glycol molecular weight and concentration. *Somatic Cell Genetics* **2**: 271-80.

Di Berardino D and Iannuzzi L. 1982. Detailed description of R-banded bovine chromosomes. *Journal of Heredity* **73**: 434-38.

El-Nahas S M, de Hondt H A, Othman O S, Bosma A A and de Haan N A. 1993. Assignment of genes to chromosome 4 of the river buffalo with a panel of buffalo-hamster hybrid cells. *Journal of Animal Breeding and Genetics* **110**: 182-85.

Gefter M L, Margulies D H and Scharff M D. 1977. A simple method of polyethylene glycol-promoted hybridization of mouse myeloma cells. *Somatic Cell Genetics* **3**: 231-36.

Goding J W. 1983. *Monoclonal Antibodies: Principles and Practices.* Pp 56-97. Academic Press, New York.

Hallerman E M, Theilmann J L, Beckmann J S, Soller M and Womack J E. 1988. Mapping of bovine prolactin and rhodopsin genes in hybrid somatic cells. *Animal Genetics* **19**: 123-31.

Iannuzzi L. 1994. Standard karyotype of the river buffalo (*Bubalus bubalis* L. 2n=50). Report of the committee for the standardization of banded karyotypes of the river buffalo. *Cytogenetics and Cell Genetics* **67**: 102-13.

Joshi C G. 1995. 'Identification and assignment of syntenic group to chromosome using interspecific cell hybrids in buffalo'. Ph.D. Thesis Submitted to Tamil Nadu Veterinary and Animal Sciences University, Madras.

Joshi C G, Rank D N, Kanakaraj P, Thiagarajan V and Krishnan A R. 1995. Interspecific somatic cell hybridization studies between buffalo fibroblast and RAG cells. *Indian Veterinary Journal* **72**: 66-70.

Norwood T H, Zeigler C J and Martin G M. 1976. DMSO enhances PEG mediated somatic cell fusion. *Somatic Cell Genetics* **2**: 263.

Olsson L and Kaplan H S. 1983. Human-human monoclonal antibody-producing hybridomas: Technical aspects. *Methods in Enzymology* **92**: 3-16.

O'Malley K A and Davidson R L. 1977. A new dimension in suspension fusion techniques with polyethylene glycol. *Somatic Cell Genetics* **3**: 441-48.

Pontecorvo G. 1975. Production of mammalian somatic cell hybrids by means of polyethylene glycol treatment. *Somatic Cell Genetics* **4**: 397-400.

Rank D N. 1996. 'Identification of gene synteny using somatic cell hybrids in river buffalo (*Bubalis bubalis*)'. Ph.D. Thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Madras.

Rank D N, Joshi C G, Kanakaraj P, Brahmkshtri B P, Kharadi V B, Nainar A M and Krishnan A R. 1998. Somatic cell hybridization between buffalo and mouse cells. *Indian Journal of Animal Sciences* **68**(9): 991-92.

Ryttman H, Thebo P, Gustavsson I, Gahne B and Juneja R K. 1986. Further data on chromosomal assignements of pig enzyme loci LDHA., LDHB, MPI, PEPB and PGM1 using somatic cell hybrids. *Animal Genetics* **17**: 323-33.

Saidi-Mehtar N, Hors-Cayla M C and van Cong N. 1981. Sheep gene mapping by somatic cell hybridization: Four syntenic groups: ENO1 - PGD, ME1-PGM3, LDHB-PEPB-TPI and G6PD-PGK-GALA. *Cytogenetics and Cell Genetics* **30**: 193-204.

Sparkes R S, Mohandas T, Sparkes M C and Shulkin J D. 1978. Aconitase (E.C.4.2.1.3) mitochondrial (ACONM) mapped to human chromosome 22. *Cytogenetics and Cell Genetics* **22**: 226-27.

Womack J E and Moll Y D. 1986. Gene map of the cow: conservation of linkage with mouse and man. *Journal of Heredity* **77**: 2-7.

Indian Journal of Animal Sciences 73 (10): 1141-1143, October 2003

Relationships between heart girth and body weight measurements of South African indigenous Nguni goats

A A AKINGBADE[1], I V NSAHLAI[2] and C D MORRIS[3]

Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Received: 5 September 2002; Accepted: 15 June 2003

ABSTRACT

This study was aimed at examining the relationships between various paired parameters of gravid pregnant South African indigenous Nguni goats and their products of pregnancy. Data were obtained from 15 pregnant does (mature female goats) and 28 kids (newborns). Heart girths and body weights of dams at mating, kidding and weaning periods were positively (0.90, 0.85 and 0.80, respectively) and significantly (P<0.001) correlated. Birth weights and heart girths of kids at birth and weaning were positively (r=0.25 and 0.85 respectively) and significantly (P<0.001) correlated. The use of birth type (litter size) to predict kids weaning weights was reliable (r=0.60; P=0.001). Also, dams mating weight was a good predictor (r=0.51; P=0.006) of kids birth weight, while correlation coefficient between dams kidding weights and kids birth weights was positive and significant (r=0.48; P<0.01). The good correlation between parameters of dams and kids between mating and kidding perhaps was because of total dependence of foetus(es) on maternal nourishment during gestation. The use of dams kidding weights to predict kids weaning weighs was unreliable (r=0.08; P=0.686). Similarly, correlation between dams and kids body weights at weaning was poor (r=0.19; P=0.341). These poor and unreliable relationships of kids perhaps were due to less dependence of kids on maternal nourishment (milk) as they age and able to survive on adult feeds.

Key words: Goats, Heart girth, Live weight, Litter size

Statistically, to comment on the quality of relationship between 2 different things, there is a need to subject both to regression analysis which explains the extent to which the relationship of one is explained by its relationship with the other (Roden 1993). Correlation studies between body measurements and body weight (Poti and Bedo 1999), various milk nutrient and mineral element constituents (Park and Chukwu 1989), milk yield and dams live weight, dams live weight and kids pre-weaning body weights (Bedo *et al.* 1998), body weights of kids at various stages of pre-weaning (Costa *et al.* 1995), live weight of newborns and dams at parturition (Tomar *et al.* 1997), were studied. From the search of literature there were no reported studies on regression analysis of parameters in pregnant South African indigenous Nguni goats and their products of gestation.

Indigenous animals worldwide constitute valuable sources of genetic materials because of their adaptation to the harsh local climatic and nutritional constraints (Cronje 1998) and are mostly found in rural areas where they are kept mainly for household consumption, source of income, ceremonies and cultural practices (Orskov and Viglizzo 1944, Mkhwanazi 1997). Growth performance can be assessed using a weighing scale, however, the cost and the small size of stock kept by each household in a rural set up make it un-affordable and un-economic to acquire by peasants that constitute vast majority of indigenous livestock owners.

Varade *et al.* (1997) reported that body measurement notably heart girth of some animal breeds can be used to predict body weight in the absence of weighing scale in rural areas. However, absence of any reported studies on regression analysis of heart girth and body weight of pregnant South African indigenous Nguni goats (SAING) and their products of gestation prior to weaning has made it impossible to know whether heart girths of the breed can also adequately serve to determine live weight. This study was expected to assess the reliability of heart girth measurement in predicting body weight of Nguni goat breed.

MATERIALS AND METHODS

Pregnant does (15) and 28 kids were used for the study.

Present address: [1]Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

[2]Discipline of Animal and Poultry Science, University of Natal, Pietermaritzburg, South Africa.

[3]Range and Forage Institute, Agricultural Research Council, Pietermaritzburg, South Africa.

The description of Nguni goat breed, reproductive and grazing management practices employed were described by Akingbade *et al.* (2001). The does were grazed solely on *Leucaena leucocephala*-grass (LGP) during gestation and were offered concentrate diet containing 20.6% crude protein (CP) as supplement and drinkable water during lactation in their night camps between 15.00 and 07.00 hr.

Throughout the study, kids were not allowed access to the grazing field; heart girth and body weight of the does were taken at mating and subsequent measurements every fortnight throughout gestation. At kidding, heart girth and body weight of dams and their respective kids were measured and subsequent measurements were taken every fortnight over a 10 week period.

Data of various parameters were appropriately paired and subjected to regression analysis using the Minitab statistical package (Minitab 1998).

RESULTS AND DISCUSSION

The correlations of heart girths and body weights dams at mating, kidding and weaning were 0.90,0.85 and 0.80, respectively ($P<0.001$; Table 1). Varade *et al.* (1997) reported that heart girths and body weights of adults were significantly and positively correlated. Birth weights and heart girths of kids at birth and weaning were significantly ($P<0.001$) and positively correlated ($r=0.25$ and 0.85, respectively). Nigm *et al.* (1995) reported that heart girth tended to be the best predictor of body weight. The correlation coefficient of relationship between litter size and weaning weights of kids was also positive and significant ($r=0.60$; $P<0.001$) and agrees with the reports of Gibb and Teacher (1982) and Husain *et al.* (1996) that litter size influenced pre-weaning growth performance.

Dams body weights at mating and kids body weights at kiding were significantly and positively correlated ($r=0.51$; $P=0.006$); correlation coefficient between dams body weights at kidding and those of their kids at birth was 0.48 ($P<0.01$) and concurs with a previous report (Tomar *et al.* 1997). In this study, heart girths of pregnant does and their products of pregnancy were significantly and positively correlated at various reproductive stages (mating, kidding and weaning); the trend probably was due to total dependence of foetus (es) on maternal nourishment during gestation. However, using dams body weights at kidding to predict weaning weights of kids, was less reliable ($r=0.08$; $P<0.686$). Similarly, correlation coefficient of the relationship between dams and kids body weights at weaning was comparatively low and insignificant ($r=0.19$; $P< 0.341$). The poor relationship probably was due to the effect of nutrition on kids pre-weaning growth. Differences in dams ability to mobilise body reserves for milk production or less dependence of kids on maternal nourishment as they age might have influenced their growth potential and possibly accounted for the poor correlation.

Table 1. Regression analysis of heart girth and live weight measurements of South African indigenous Nguni goats

Periods/parameters compared	LGP	P-values
Does		
Mating	MHG = 54.8+0.717MWt	0.001
Does mating heart girth vs does mating weight	r = 0.90	
Kidding	KHG = 63.2+0.494KWt	0.001
Does kidding heart girth vs does kidding weight	r = 0.85	
Weaning	WHG = 61.6+0.452WWt	0.001
Does weaning heart girth vs does weaning weight	r = 0.80	
Kids		
Birth	KBHG = 18.3+4.20KBW	0.001
Kids birth wt. vs kids birth heart girth	r = 0.25	
Weaning	KWHG = 29.9+1.73KWW	0.001
Kids weaning wt. vs kids weaning heart girth	r = 0.85	
Litter size vs kids weaning wt.	KWW = 12.4 - 1.15LS	0.001
	r = 0.60	
Does and kids		
Dam mating wt. vs kids birth wt.	KBW = 1.57+0.0207DWM	0.006
	r = 0.51	
Dam kidding wt. vs kids birth wt.	KBW = 1.55+0.0181DWK	0.010
	r = 0.48	
Dam kidding wt. vs kids weaning wt.	KWW = 8.91+0.0160DWK	0.686
	r = 0.08	
Dam weaning wt. vs kids weaning wt.	KWW = 7.86+0.424DWW	0.341
	r = 0.19	

Most correlation coefficients of heart girth vs body weights of adults (does) in this study were higher than for kids in line with the report of Barari *et al.*(1999) that predictions were more accurate for calves than adult yaks. Though statistical results from the various parameters compared in this study could be very valuable in estimating body weight of kids, nutrition during pre-weaning period most likely explains the poor correlation between dams kidding weight and weaning weights and those of their kids at weaning.

The results from this study extend support to previous studies by showing that heart girths of Nguni breed can also be used to predict body weights especially in rural areas known to lack weighing apparatus.

ACKNOWLEDGEMENTS

This is a collaborative study between the Disciplines of Animal amd Poultry Science and Grass Land Science, University of Natal, South Africa. This project was partly funded by the National Research Foundation (NRF) and the Range and Forage Institute of the Agricultural Research Council of South Africa.

REFERENCES

Akingbade A A, Nsahlai I V, Bonsi M L K, Morris C D and Du Toit L P. 2001. Reproductive performance of South African indigenous goats inoculated with DHP-degrading rumen bacteria and maintained on *Leucaena leucocephala*/grass mixture and natural pasture. *Small Ruminant Research* **39**(1): 73-85.

Barari S K, Basu A, Nandankar U A and Mohanty T K. 1999. Estimation of body weight of yak. *Indian Journal of Animal Sciences* **69**(5): 352-54.

Bedo S, Nicodemusz E, Nagy Z, Seregi J and Kukovics S. 1998. Milk urea and lactose as indicators of the protein and energy status in lactating ewes and goats. Sheep and goat production in Central and Eastern European countries. *Proceedings of the Workshop, Budapest, Hungary, REU Technical Series,* No. 50, pp. 204-11.

Costa R G, Pimenta-Filho E.C, Santos E S and Ribeiro M N. 1995. Correlations between pre-weaning body weights of crossbred Anglo-Nubian kids. *Ars Veterinaria* **11**(1) : 66-70.

Cronje P B. 1998. *Perspectives on the Constraints, opportunities and Issues Surrounding research on Goat Production in South Africa.* pp. 2-5. (Eds) Webb E C and Cronje P B. co-published with University of Fort Hare and University of Sydney.

Gibb M J and Treacher T T. 1982. The effect of body condition and nutrition during late pregnancy on the performance of grazing ewes during lactation. *Animal Production* 34 *: 123-29.*

Husain S S, Horst P and Islam A B M M. 1996. Study on the growth performance of Black Bengal goats in different periods. *Small Ruminant Research* **21**(3): 165-171.

Minitab Software Statistical Package. 1998. Release 12.1, WNN1210.01917, Minitab Inc. 814-238-3280.

Mkhwanazi N. 1997. *Goats for Cultural Purposes.* pp. 67-72. (Ed) Brigid L. Cedara Report Goat Production. Cedara Report No. N/A/98/5.

Nigm A A, Abdalla O M, Aboul-Ela M B, Kamel H M and Ahmed M A. 1995. Meat characteristics of sheep and goat breeds commonly consumed in UAE. 2. Use of body dimensions for predicting body and carcass weights. *Emirates Journal of Agricultural Sciences* **7**: 39-54.

Orskov E R and Viglizzo E F. 1994. The role of animals in spreading farmers' risks: a new paradigm for animal science. *Outlook in Agriculture* **23**: 81-9.

Park Y W and Chukwu H I. 1989. Trace mineral composition in goat milk from French-Alpine and Anglo-Nubian breeds during the first five months of lactation. *Journal of Food Composition and Analysis* **2**(2): 161-69.

Poti P and Bedo S. 1999. Data on body measurements of various sheep breeds. Future prospect for Hungarian animal production (challenges and opportunities). Scientific conference at the Hungarian Academy of Sciences. *Allattenyesztes es Takarmanyonas* **48**(6): 732-33.

Roden J. 1993. 'Statistical Analysis Handbook.' M Sc. Animal Nutrition Programme, University of Aberdeen, Aberdeen, U.K.

Tomar A K S, Kumar A and Singh N P. 1997. Pattern of birth weight inheritance in Sirohi and Kutchi goat breeds in semi-arid conditions of western India. *Indian Journal of Small Ruminants* **3**(1): 1-4.

Varade P K, Zli S Z and Malkhede P S. 1997. Bodey measurements of local goats under field conditions. *Indian Veterinary Journal* **74**(5): 448-49.

Indian Journal of Animal Sciences 73 (10): 1144-1146, October 2003

Effects of milk yield determination on growth performance of South African indigenous Nguni goat kids

A A AKINGBADE[1], I V NSAHLAI[2] and C D MORRIS[3]

Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Received: 29 January 2001; Accepted: 26 June 2003

ABSTRACT

This study examined the effects of combination of weigh-suckle-weigh and hand milking method of milk yield determination on growth performance of kids during the first 4 weeks of birth when they are solely dependent on dams for survival. The experimental treatments comprised milked and un-milked treatments. Kids birth weights and their weights at 28 days of age were positively and significantly correlated (milked treatment: r=0.71; P<0.001; un-milked treatment: r=0.54; P<0.058), but correlation coefficient of regression analysis between birth type and kids weight at 28 days of age was not significant (milked: r=0.34; P>0.05; un-milked: r=0.37; P>0.05). Between kidding and day 28 of the study, un-milked dams lost 1.48kg (-3.94 vs -2.46; P>0.05) more body weight than that of milked dams. The marginally higher body weight loss of un-milked dams was perhaps mobilised towards milk synthesis,as their kids were consistently heavier (P>0.05) and gained 580g (2.34±1.33 vs 1.76±0.83 kg; P>0.05) more body weight than that of their counterparts nursed by dams that were milked. The results from the study seems to imply that combination of weigh-suckle-weigh and hand milking method was not stressful to kids and dams; the method had no adverse effects on pre-weaning live weight changes of both kids and dams.

Key words: Body weight, Goats, Milk

Kids are provided colostrum for immunity (Rattray 1992) and milk for maintenance. Most pre-weaning kid mortalities occur during the first 3 to 4 weeks of lactation (Treacher 1983) when kids are solely dependent on maternal nourishment for immunity, maintenance and growth. Daily body weight gains of kids during the first 4 weeks of life are highly correlated to milk consumption (Geenty and Dyson 1986) and dams milk production (Gibb and Treacher 1978).

Methods of milk determination include hand milking without oxytocin injection, hand milking with oxytocin injection and combination of weigh-suckle-weigh and hand milking methods (Aboul-Naga *et al.* 1981, Geenty 1985). There are many reported studies on methods of milk yield determination in ruminant animals. However, there were no studies that examine the effects of milk yield determination on growth performance of suckling kids.

The objective of the study was to examine the effects of milk yield determination on the growth performance of kids of South African indigenous Nguni goats.

Present address: [1]Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

[2]Discipline of Animal and Poultry Science, University of Natal, Pietermaritzburg, South Africa.

[3]Range and Forage Institute, Agricultural Research Council, Pietermaritzburg, South Africa.

MATERIALS AND METHODS

South African indigenous Nguni does and kids were used for the study. The experimental treatments comprised milked and un-milked treatments. Milked treatments had 11 lactating dams and nursed 17 kids while un-milked treatments had 9 lactating dams and 13 kids. Experimental diet was *Leucaena leucocephala*-grass pasture, but dams on milked treatment were fed solely on concentrate diet containing 14.8% crude protein on each day of milk yield determination. Mineral supplements were not given during the study. Dams and kids were weighed within 48 hr of birth and then on a weekly basis during the first 4 weeks of lactation.

Milk yield was determined using combination of the weigh-suckle-weigh and hand milking methods (Aboul-Naga *et al.* 1981). Milk yield was measured a day per week and weekly for 4 weeks, and commenced during the first week of lactation. At 18.00hr of the day preceding the milk evaluation, the kids were separated from the dams and the udder was completely drained by hand. At 08.00, 13.00 and 17.00hr on sampling days the kids were weighed, returned to their respective dams and allowed to suckle for 30 min, then were removed from their dams and weighed again and seperated from their dams until the next sampling period. The remaining milk in the udder was drained by hand and weighed. The daily total milk yield was taken as the difference in weight of

kids before and after suckling plus the respective weights of residual milk obtained during hand milking from the udder of the respective dams. The weekly milk yield of each doe was deduced by multiplying her total milk yield on the day of milk sampling by seven.

Growth performance of kids was evaluated by weighing them on a weekly basis throughout the study. The growth performance of kids was monitored not beyond the first 4 weeks of lactation as previous report on milk yield determination (Coombe *et al.* 1960) has shown that beyond 4 weeks, accurate measurement of body weight gain due to milk consumption becomes difficult.

Forage species on LGP plot were sampled for analysis as per Akingbade *et al.* (2001). Diets were analysed for dry matter (DM), crude protein (CP), ether extract (EE) and ash (AOAC 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to Van Soest *et al.* (1991).

Data were subjected to regression analysis and analysis of variance (ANOVA) using the following general linear model (GLM) of Minitab statistical package (Minitab 1998):
Birth weight of kids

$$Y_{ijkl} = U + T_i + BT_j + S_k + D_l + e_{ijkl}.$$

Pre-weaning live weight change of kids

$$Y_{ikpn} = U + T_i + S_k + D_p + B_n + e_{ikpn}.$$

Live weight of does at kidding

$$Y_{ijl} = U + T_i + BT_j + D_l + e_{ijl}.$$

Where Y_{ijkl}, Y_{ikpn} and Y_{ijl} = individual observations; U =overall mean; T_i = effect of treatment; BT_j = effect of birth type; B_n = effect of birth weight; S_k = effect of gender; D_l = effect of dam weight at mating; D_p = effect of dam weight at parturition; e_{ijkl}, e_{ikpn} and e_{ijl} = unexplained variation assumed to be randomly and independently distributed. Means of treatments were compared using a t-test (Steel and Torrie 1960).

RESULTS AND DISCUSSION

Proximate composition of forage resource on LGP and the concentrate diet fed to milked dams on each day of milk yield determination is presented on Table 1.

The correlation coefficient of kids birth weights vs kids weights at 28 days of age on milked ($r = 0.71$; $P < 0.001$) and un-milked ($r = 0.54$; $P = 0.058$) groups were positive and significant (Table 2). However, relationships between kids birth types and kids weights (milked treatment: $r = 0.34$, $P > 0.05$ and un-milked treatment $r = 0.37$, $P > 0.05$) and that between dams weights and kids weights (milked treatment: $r = 0.18$, P. 0.05 and un-milked treatment: $r = 0.24$, $P > 0.05$) on day 28 of the study were not significantly correlated.

Between kidding and 28 days post kidding, un-milked dams lost 1.48 kg (-3.94vs- 2.46, $P > 0.050$) more body weight than that of milked dams. The slightly higher body weight loss of un-milked dams perhaps was mobilised towards milk synthesis (Crosse *et al.* 1998), as their kids were consistently heavier ($P > 0.05$) and gained 580g (2.34±1.33 vs 1.76±0.83kg; $P > 0.05$) more body weight than that of kids nursed by dams on milked treatment. Gibb and Treacher (1978) and Geenty and Dyson (1986) showed that dams milk yield influenced early growth performance of kids and differences in growth rates were associated with differences in milk consumption (Gibb and Treacher 1980).

Table 1. Nutrients and minerals of forage from *Leucaena leucocephala*-grass pasture (LGP) and concentrate diet

Nutrients and minerals	LGP	Concentrate
% DM		
Moisture	3.2	9.0
Crude protein	25.8	14.8
Neutral detergent fibre (NDF)	29.2	35.9
Acid detergent fibre (ADF)	19.0	16.7
Fat	3.0	2.5
Ash	9.4	6.4
g/kg DM		
Calcium (Ca)	30.5	48.0
Phosphorus (P)	15.1	80.0
Magnesium (Mg)	14.8	26.0
mg/kg DM		
Zinc (Zn)	370	16
Iron (Fe)	3980	107
Copper (Cu)	130	256

Kids weekly live weights and their weight gains between birth and 28 days post birth on both treatments were not significant ($P > 0.05$; Fig. 1). The insignificant differences between treatments in kids body weight gains and live weight

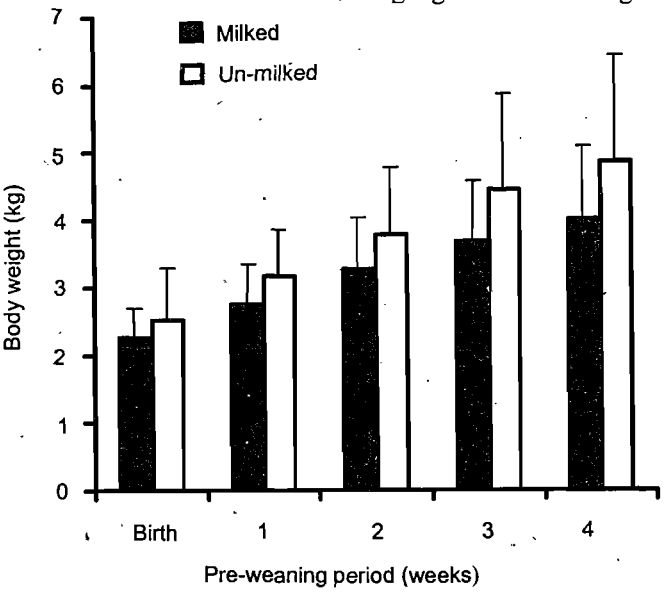


Fig. 1. Pre-weaning growth rate of South Africa indigenous Nguni goat kids.

Table 2. Relationships between birth type and body weights of South African indigenous Nguni goat kids and dams

Parameters paired	Milked treatment	P-values	Un-milked treatment	P-values
Kid birth wt vs Kids wt wk 4	KWW4 = 0.09+1.75KBW r = 0.71	0.001	KWW4 = 2.05+1.12KBW r = 0.54	0.058
Birth type vs Kids wt wk 4	BT = 3.18 - 0.251KWW4 r = 0.34	0.187	BT = 2.60 - 0.170KWW4 r = 0.37	0.215
Dams wt. wk 4 vs Kids wt wk 4	KWW4 = 3.35+0.0177DWW4 r = 0.18	0.499	KWW4 = 3.61+0.0311DWW4 r = 0.24	0.418

changes of dams during the study suggested that combination of weigh-suckle-weigh and hand milking method of milk yield determination was not stressful to both kids and dams on milked treatment and that the milked treatment had no adverse effects on pre-weaning growth of kids and dams live weight changes.

Though studies of Gibb and Treacher (1978), and Geenty and Dyson (1986) attributed early growth performance of kids to milk yield of dams, the milked treatment revealed that the quantity of milk consumed by kids rests on the mothering ability of dams (i.e. willingness of their dams to stand to be suckled) and also on the kids ability (vigour) to extract maximum quantity of milk within the time frame allowed by dams.

Our results showed that growth performance of kids and pre-weaning live weight changes of dames subject to combination of weigh-suckle-weigh and hand milking method of milk yield determination were not adversely affected, and thus could serve to allay the fear in minds that always restrict the use of such animals for scientific research. However, this result cannot be said to apply for other methods of milk determination. Future studies should consider the growth performance of kids and pre-weaning live weight changes of dams using other methods of milk yield determination that were not covered in this study.

ACKNOWLEDGMENTS

This was a collaborative study between the Disciplines of Animal and Poultry Science and Grassland Science. University of Natal, South Africa. This project was partly funded by the National Research Foundation (NRF) and the Range and Forage Institute of the Agricultural Research Council of South Africa.

REFERENCES

Aboul-Naga A M, EL-Shobokshy A S and Moustafa M A. 1981. Milk production from sub-tropical non-dairy sheep. 2. Method of measuring. *Journal of Agricultural Science (Cambridge)* **79**: 303-08.

Akingbade A A, Nsahlai I V, Bonsi M L K, Morris C D and du Toit L P. 2001. Reproductive performance of South African indigenous goats inoculated with DHP-degrading rumen bacteria and maintained on *Leucaena leucocephalal*/grass mixture and natural pasture. *Small Ruminant Research* **39**(1); 73-85.

AOAC. 1990. *Official Methods of Analysis*. 15th edn. Association of Official Analytical Chemists, Inc., Washington DC, USA.

Coombe J B, Wardrop I D and Tribe D E. 1960. A study of milk production of the grazing ewe, with emphasis on the experimental technique employed. *Journal of Agricultural Science* **54**: 353-59.

Crosses S, Umunna N N, Osuji P O, Tegegne A, Khalili H and Tedla A. 1998. Comparative yield and nutritive value of forages from two cereal-legume based cropping systems. 2. Milk production and reproductive performance of crossbred dairy cows. *Tropical Agriculture (Trinidad)* **75**(4): 415-21.

Geenty K G. 1985. Lactation performance, growth, and carcass composition of Sheep. 2. Relationships between ewe milk production, lamb water turnover, and lamb growth in Romney, Dorset, and crossbred sheep. *New Zealand Journal of Agricultural Research* **28**:249-55.

Geenty K G and Dyson C B. 1986. The effects of various factors on the relationship between lamb growth rate and ewe milk production. *Proceedings of New Zealand Society of Animal Production* **46**: 265-69.

Gibb M J and Treacher T T. 1978. The effect of herbage allowance on herbage intake and performance of ewes and their twin lambs grazing perennial ryegrass. *Journal of Agricultural Science (Cambridge)* **90**: 139-47.

Gibb M J and Treacher T T. 1980. The effect of ewe body condition at lambing on the performance of ewes and their lambs at pasture. *Journal of Agricultural Science (Cambridge)* **95**: 631-40.

Minitab software statistical package, 1998. Release 12.1, WNN1210.01917, Minitab Inc. 814-238-3280.

Rattray P V. 1992. Nutrition of the ewe during gestation and lactation progress. *Sheep and Goat Research*. pp. 85-105. (Ed) Speedy A W. C A B International. 1992. Wallingford. Oxon OX10. 8DE. UK.

Steel R G D and Torrie J H. 1960. *Principles and Procedures of Statistics*. McGraw-Hill Book Co., New York.

Treacher T T. 1983. Nutrient requirements for lactation in the ewe. *Sheep Production*. pp. 133-53. (Ed) Haresign W. Butterworths, London.

Van Soest P J, Robertson J B and Lewis B A. 1991. Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3582-597.

Indian Journal of Animal Sciences **73** (10): 1147-1149, October 2003

Growth and reproductive performance of Magra breed of sheep

S C MEHTA[1], V K SINGH[2], M AYUB[3] and V MEHROTRA[4]

Central Sheep and Wool Research Institute, Arid Region Campus, Bikaner, Rajastan 334 006 India

Received: 1 January 2002; Accepted: 5 June 2003

ABSTRACT

The growth and reproductive performance of Magra sheep were studied. The body weights birth at 3, 6, 9 and 12 months were 2.75±0.03, 13.43 ± 0.17, 17.86±0.24, 20.38±0.25 and 24.84±0.37 kg respectively. The effect of year was highly significant. The spring born animals were heavier than those born in the autumn. The weight gain/day from birth to 3 months, 3 to 6 months, 6 to 9 months and 9 to 12 months was observed to be 118.67, 49.22, 28 and 49.56 g respectively. The tupping, lambing on available basis and lambing on bred basis were 52.2, 41.3, 79.0 and 77.1,65.8, 85.4%, respectively, for the year 1998 and 1999.

Despite high temperature, very less relative humidity and very low rainfall resulting in reduction of vegetation in the pasture land and causing physiological stress, this breed performed well and hence can be used for the genetic improvement in the farmers' flock for the non-migratory or short distance migratory flocks of sheep for better quality and production of wool and meat.

Key words: Growth, Mutton, Reproduction, Sheep

Magra breed of sheep is well known for the production of best carpet quality wool. This breed inhabits mainly the eastern and southern parts of Bikaner district in Rajasthan. Animals of this breed belonging to certain pockets in the breeding tract are also designated as "Bikaneri Chokla" due to the production of most lustrous wool (Acharya 1982, Basuthakur 1988). This is perhaps the best breed available in Rajasthan as it not only produces lustrous wool but the quality of wool produced (Mehta *et al.* 1998) and the growth of lambs, in terms of body weight, is higher in this breed as compared to most of the breeds of Rajasthan (Annual Reports, CSWRI 1990-2000).

The climate of the breeding tract of this breed is arid with an average annual rainfall of 260 mm. The mean maximum and minimum temperature remains 42°C and 5°C, respectively. With the change in overall scenario of textile industry where synthetic fibres have replaced natural fibres to a great extent for variety of purposes such as, manufacture of winter season clothes, casuals, hosiery items etc., the cost of wool has not increased proportionately and the wool has become secondary commodity in sheep husbandry. Under this changed scenario the maximum return to a sheep farmer is through the sale of surplus animals for mutton (Mehta *et al.* 1995). The reproductive efficiency of the flock has direct bearing on the economic returns to a sheep farmer because it is directly proportional to the number of animals in the flock and number of animals available to a sheep farmer for sale. This study was therefore taken up to evaluate the growth and reproductive efficiency of flock at an organized farm.

Present address: [1]Scientist, National Research Centre on Camel, Post Box-07, Bikaner.

[2]Director. [3-4]Technical Officer, Central Sheep and Wool Research Institute, Arid Region Campus, Bikaner-334006

MATERIALS AND METHODS

Data were collected from the Magra flock maintained at the Central Sheep and Wool Research Institute, Arid Region Campus, Bikaner, from the year 1998 to 2000. At the Institute, this flock was initiated with the purchase of male lambs of about 3 months age in March 1996. This was followed by the purchase of 180 females and 5 males of 10-12 months age in the October 1997. The rams were selected for breeding on the basis of phenotype only. Females were mated at random to the selected males and growth performance of their progeny was recorded. The reproductive efficiency of the ewes from spring 1998 to autumn 2000 was also recorded.

Least-squares maximum likelihood programme (Harvey 1987) was used to study the effect of various genetic and non-genetic factors on growth. All effects were taken as fixed.

RESULTS AND DISCUSSION

The least-squares means of Magra lambs from birth to 12 months of age are presented in Table 1. The birth, 3, 6, 9 and 12 months body weights were 2.75±0.03, 13.43 ± 0.17, 17.86±0.24, 20.38±0.25 and 24.84±0.37 kg respectively.

Table 1. Growth of Magra lambs (weight in kg)

Effects	Birth Wt.	3M Wt.	6M Wt.	9M Wt.	12M Wt.
μ	2.75±0.03	13.43±0.17	17.86±0.24	20.38±0.25	24.84±0.37
	(313)	(286)	(247)	(225)	(141)
Year	**	**	**	**	**
1998	2.82±0.08	17.34±0.49	21.63±0.65	24.76±0.68	27.82±0.74
	(21)	(21)	(21)	(21)	(21)
1999	2.90±0.03	13.71±0.21	18.64±0.28	19.72±0.30	21.85±0.32
	(151)	(146)	(138)	(121)	(120)
2000	2.52±0.04	8.80±0.29	13.30±0.41	16.67±0.44	-
	(141)	(119)	(88)	(83)	
Sex	**	**	**	**	**
Male	2.81±0.03	13.80±0.21	18.59±0.29	21.25±0.31	26.20±0.46
	(165)	(154)	(124)	(109)	(67)
Female	2.70±0.03	13.06±0.22	17.13±0.29	19.51±0.31	23.48±0.43
	(148)	(132)	(123)	(116)	(74)
Season of birth	NS	NS	*	**	*
Spring	2.78±0.04	13.37±0.24	18.42±0.32	21.60±0.35	25.67±0.54
	(159)	(233)	(194)	(174)	(91)
Autumn	2.72±0.05	13.49±3.30	17.30±0.39	19.16±0.42	24.01±0.43
	(54)	(53)	(53)	(51)	(50)
Regression	Dwtl**	Bwt **	Bwt **	Bwt **	Bwt **
coefficient	0.05±0.006	1.62±0.54	2.87±0.48	3.22±0.54	2.65±0.78
Average	28.87±0.19	2.92±0.03	2.79±0.03	2.79±0.03	2.91±0.03

NS., Nonsignificant, *(P<0.05), **(P<0.01).

Rajasthan had third consecutive drought in the year 2000, the annual rainfall has gone down from 475.7 mm in the year 1998-99 to 178.5 and 196.7 mm, respectively, in the year 1999-2000 and 2000-2001 with significant reduction in average maximum and minimum relative humidity from 62.23 and 35.58% in the year 1998-99 to 54.83 and 23.17% in the year 1999-2000 and 52.33 and 24.17% in the year 2000-2001 (Meteorology Department, Government of Rajasthan) which is clearly reflected in the year-wise means of the body weights. The effect of year due to this main reason was highly significant (P< 0.01). The growth performance of the flock was better in the initial year 1998 and it declined sequentially in the year 1999 and 2000. The effect of the sex was highly significant (P< 0.01) with superiority of the males over females. Spring born animals were heavier than those born in the autumn (P < 0.01). The body weights reported by Acharya (1983) are almost similar to those obtained for the year 1998 in this study, indicating that the growth performance of the flock should have been even better, if there would have been no drought. The weight of the milk teeth animals of this breed belonging to farmers flock has been reported to be 25.61 ±0.16 kg in the year 1995 -96 (Annual Report, Network Project on Sheep Improvement 1995-96). This report can also be said to be consistent with the present report while taking into consideration the effect of drought.

The per day gain in the weight from birth to 3 months, 3 to 6 months, 6 to 9 months and 9 to 12 months was observed to be 118.67, 49 22, 28 and 49.56g respectively (Fig. 1). Keeping in view the drought conditions, if the per day gain is viewed for each year separately then it becomes quite clear that when the availability of feed and fodder is sufficient enough then a sheep farmer can sale his surplus animals at any time after 6 months of age with the option that "sooner is better". However, when the initial growth is slow due to drought or any such reason, except health, then it may at times be better to wait till 12 months of age because there remains enough scope for the improvement of body weight as it has to go far to reach the optimum weight for the defined age. With little favour, either by nature or by improved feeding management, the weight gain may be substantial. In present case, the weight gain/day was 12 g from 6 to 9 months of age in the year 1999, the same has increased to 23.67 g from 9 to 12 months of age.

Table 2. Reproductive performance of Magra ewes

Season/ year	Ewes available	Ewes bred	Ewes lambed	Tupping (%)	Lambing% Available	Lambing% Bred
Spring 98	180	40	21	22.2	11.7	52.5
Autumn 98	157	136	118	86.6	75.1	86.8
Pooled 98	357	176	139	52.2	41.3	79.0
Spring 99	82	46	33	56.1	40.2	71.7
Autumn 99	184	159	142	86.4	77.2	89.3
Pooled 99	266	205	175	77.1	65.8	85.4
Autumn 2000	245	188	161	76.73	65.71	85.64

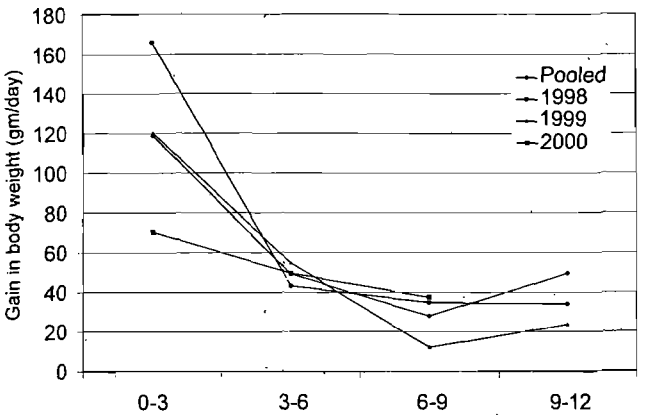


Fig 1. Per day gain in body weight of Magra lambs.

The regression of dam's weight on birth weight of lambs and that of birth weight on all subsequent weights was significant ($P<0.01$) indicating the usefulness of birth weight for early selection of the animals for growth.

The reproductive performance of the Magra ewes from spring 1998 to autumn 2000 is presented in Table 2. In the spring 1998, only 40 ewes out of 180 could be bred because these females were purchased in October 1997 at the age of 10-12 months. Not all females come in oestrous at 18 months of age. Further the spring season is a minor breeding season for sheep. In spring 1999 the tupping was 56.1%. The reproductive performance in autumn season was good enough in the year 1998 and 1999, where the tupping, lambing on available basis and lambing on bred basis were 86.6, 75.1, 86.8 and 86.4, 77.2, 89.3%, respectively, for the two years. In the year 2000 the per cent tupping was relatively low (76.73%) as compared to the previous 2 years probably due to the impact of drought. However, the lambing on available basis was comparable i.e. 85.64%.

Since the data belongs to an organised farm the impact of drought has been reduced to a great extent but there still remains enough scope for the achievement of 100% lambing in a year. To achieve this, apart from feeding dry fodder and concentrate, sufficient green fodder should be made available to the animals. Despite high temperature, very less relative humidity and very low rainfall resulting in reduction of vegetation in the pasture land and causing physiological stress, this breed performs well. There exists lot of variation, which can be used in selective breeding for further improvement of this breed. At present status of production also this breed is superior to majority of the indigenous carpet wool producing breeds and hence can be used for the genetic improvement in the farmers' flock for the non-migratory or short distance migratory flocks of sheep for better quality and production of wool and meat.

REFERENCES

Acharya R M. 1982. *Sheep and Goat Breeds of India.* FAO animal production and health paper 30 FAO, Rome. Italy.

Annual Reports, Central Sheep and Wool Research Institute. 1990-2000.

Annual Report, Network project on sheep improvement, RAU, Bikaner. 1995-96.

Baruthakur A K. 1988. *Sheep Research. Production and Marketing in India.* Inter-Media Publication, New Delhi, India.

Harvey W R. 1987. *User's Guide to LSMLMW PC-I Version Mixed Model Least- squares and Maximum Likelihood Computer Programme*, Mimeograph. Columbus, Ohio, USA.

Mehta S C, Singh V K, Parthasarathy S and Chopra S K. 1998. Subjective and objective lustre evaluation in Magra breed of sheep. *Indian Journal of Animal Sciences* **68** (1): 91-92.

Mehta S C, Vij R K, Nivsarkar A E and Sahai R. 1995. Sheep husbandry practices in Sonadi and Malpura breeding tract. *Indian Journal of Small Ruminants* **1**(1): 1-7.

Meteorological Department. Government of Rajasthan. 1998-2001.

Indian Journal of Animal Sciences 73 (10): 1150-154, October 2003

Mineral status of feeds and fodders in Bhiwani district of Haryana state

N S MAAN[1], A B MANDAL[2], P S YADAV[3] and P C GUPTA[4]

CCS Haryana Agricultural University, Hisar, Haryana 125004 India

Received: 8 May 2002; Accepted: 10 June 2003

ABSTRACT

Extensive survey of Bhiwani district of Haryana state was conducted from February to April. Ten representative villages, representing the whole district were selected. Sufficient number of feeds and fodders being offered to the dairy animals were collected. Records of feeding practices, disease prevalence, etc., were also collected. The samples thus collected were processed and analysed for Ca, P, Zn, Cu, Fe and Mn contents following standard procedures. The livestock were fed wheat straw, Sorghum *kadbi*, pearlmillet *kadbi*, berseem, oats and *bathua*. The concentrates were cottonseed, cottonseed-cake, mustard-cake, *guar* (clusterbean), wheat broken*dalia*, pearlmillet and complete feed pellets. None of the farmers was feeding mineral mixture, while few farmers practised feeding of common salt. The average values of Zn contents were in wheat straw 23.92±2.69, pearlmillet stover 27.52±2.76, sorghum stover 20.32±1.56, *bathua* 17.13±2.00, oats 18.08±1.37, berseem 17.03±0.82, cottonseed 46.36±3.01, cottonseed-cake 45.85±3.68, wheat broken*dalia* 31.66±2.72, pearlmillet grain 40.46±3.61, clusterbean seed 63.18±8.19 and feed pellets 35.86±3.36 ppm. On an average 4.37 to 100% feed samples had less than 40 ppm Zn. The concentrations of copper were wheat straw 1.57±0.52, pearlmillet stover 1.76±0.69 and sorghum stover 3.65±0.52, *bathua* 4.17±0.59, oats 4.70±0.76, berseem 5.47±0.78, cottonseed 12.89±0.84 and its cake 12.16±1.29, wheat broken*dalia* 10.07±0.63, pearlmillet 11.22±1.51, clusterbean seed 12.50±1.61 and feed pellets 19.80±1.92 ppm, respectively. About 0.0 to 100% of feed samples were deficient in Cu. Iron concentration in all the feeds (concentrates, dry roughages and green roughages) from both the districts was much higher than the required level. The content of Mn in wheat straw 38.25±4.80, pearlmillet stover 28.50±3.01, sorghum stover 24.22±1.08, *bathua* 37.03±3.36, oats 43.09±4.81, berseem 33.66±9.01, cottonseed 36.35±2.76, cottonseed-cake 27.92±2.95, wheat broken*dalia* 28.10±1.82 and pearlmillet 27.79±2.31, clusterbean seed 24.17±2.68 and feed pellets 21.45±3.24 ppm. The Ca and P concentrations in roughages and concentrates from both the districts were within the reported range. These feedstuffs could meet 70-100% of the requirement of both Ca and P. Majority of samples of dry and green fodders and cereal grains in this district were deficient in Zn, Cu and Mn. Cottonseed, cottonseed-cake and clusterbean seed though supplied good amount of Zn and Cu but were deficient in Mn. Fe content of feeds and fodders are quite high.

Key words: Calcium, Copper, Feeds, Fodders, Iron, Manganese, Mineral status, Nutrition, Phosphorus, Zinc

In most of the developing countries mineral deficiencies are frequently encountered in the livestock rations. Zinc deficiency is rampant both in soil and plants affecting crop yield (Kanwar and Randhawa 1967, 1974, Nayar *et al.* 1990, Dangarwala 1994). The deficiency or toxicity of minerals is an area problem. Soil mineral status keeps on changing due to pressure on land for maximum crop production, fertilizer application, rain and natural calamities, thus altering the mineral content of feeds and fodders and, hence, their supply to the animals. It is, therefore, necessary to generate zone wise information on the mineral status so as to identify deficiencies or toxicities, if any. Regional mapping of elements in feed and fodders is relatively a rapid, reliable and line data on the level of macro and microelements (Aggett *et al.* 1988). In Haryana state farmers generally do not supplement mineral mixture in animal ration, which possibly leads to many reproductive and health problems like anoestrus, delayed puberty, repeat breeding and post parturient haemoglobinuria. Keeping these points in consideration an attempt was made to assess the mineral status of feed and fodders in Bhiwani district of Haryana state.

Present address: [1]Assistant Scientist, [2]Principal Scientist, CARI, Izatnagar 243 122.

[3]Senior Scientist, [4]Formerly Senior Scientist, Department of Animal Nutrition.

MATERIALS AND METHODS

Extensive survey of Bhiwani district was conducted, from February to April. Ten representative villages/sites from each district, that represent the whole district, were selected for this purpose. The villages of Bhiwani district were Kikral, Surpura Khurd, Hui, Dhigawa, Mandoli, Bondh, Charkhi Dadri, Tosham and Bawani Khera. From each site sufficient quantity of representative sample of

feeds and fodders, which were being fed to the buffaloes by their owners, were collected following random sampling technique. The livestock owners were also approached for necessary information such as feeding practices followed then (February to April) and during the whole year, use of mineral mixture and common salt in livestock diet, history of the disease prevalence, etc. A sample of drinking water was also collected from common kholi/pond from each village.

The farmers were feeding both dry and green roughages. The concentrates being fed to animals were either home grown or procured from the market. The samples were collected in polythene bags. The samples so collected were dried in hot air oven at 100±5°C for 24 hr and ground to pass through 2 mm size sieve and stored in paper bags for laboratory analysis for minerals. The concentration of Ca, Zn, Cu, Fe and Mn in water, feeds, fodders and biological materials were estimated using atomic absorption spectrophotometer. AOAC (1984) procedure was followed for the estimation of phosphorus of feeds and fodders. Percentage of feed and fodder samples deficient in micro-minerals were calculated by comparing the individual values with the NRC (1989) specification. Moreover, the intake of all the minerals was calculated for individual animal from the records of feed offered and analyzed values of feeds and fodders, and also compared again with the NRC (1989) specifications.

RESULTS AND DISCUSSION

Bhiwani district, situated on southern part of Haryana, is a semi desert area and soil is mainly sandy. It is flanked by Hisar and Rohtak districts, which are breeding tracts of Murrah buffaloes. Most of farmers reared 1-5 milch buffaloes with good production level. Availability of greens was seasonal (i.e. during *rabi* and *kharif* seasons). During scarcity of green fodder animals are mainly fed on wheat straws and stovers (sorghum *kadbi* and pearlmillet *kadbi*). Farmers rarely conserve green fodder as hay or silage.

Mineral composition of feeds and fodders

Dry roughages: Dry roughages, viz. wheat straw, sorghum *kadbi* and pearlmillet *kadbi*, were home grown (Table 1). Wheat straw was the major dry roughage and was being fed at all the sites. Zn concentration in wheat straw ranged from 10.66 to 34.69 ppm. The concentration of zinc was lowest in Surpura Khurd (15.50±3.14 ppm) and highest in Dhigawa village (26.94±5.48 ppm). Concentration of Zn also varied within sites as standard errors ranged from 3.14 to 7.54. The pearl millet stover (Table 1) the second major dry roughage contained Zn 14.54 to 38.76 ppm on dry matter basis. Similarly the values for sorghum stover ranged from 18.41 to 32.95 ppm Zn (Table 1).

The Cu in wheat straw (Table 1) ranged from 0.00 to 7.13 ppm of DM. All the samples collected from Kadma contained undetectable level of Cu while the values collected from

Table 1. Mean concentration of minerals, their range and status in dry and green roughages

	Ca (g\100g)	P (g\100g)	Zn (µg\g)	Cu (µg\g)	Fe (µg\g)	Mn (µg \g)
Wheat straw (n=48 samples from 10 sites)						
Mean	0.23±0.02	0.08±0.01	23.92±2.69	1.57± 0.52	160.26±30.81	38.25±4.80
Range	0.14 - 0.36	0.05 - 1.12	10.66 – 34.69	0.00 – 7.13	88.01 – 277.37	10.10 – 57.22
Deficiency%	-	-	100.0	100.0	0.00	72.22
Pearl millet stover (n=14 samples from 3 sites)						
Mean*	0.32±0.02	0.09±0.01	27.52±2.76	1.76± 0.69	98.20±16.26	28.50±3.01
Range	0.26 - 0.44	0.05 - 1.13	14.54 – 38.76	0.00 –6.87	24.00 – 189.38	16.83 – 47.12
Deficiency%	-	-	100.0	100.0	18.18	81.81
Sorghum stover (n=7 samples from 2 sites)						
Mean	0.48±0.02	0.12±0.01	20.32±1.56	3.65± 0.52	108.50±4.87	24.22±1.08
Range	0.38- 0.53	0.07 - 1.15	18.41 – 32.95	0.00 – 6.25	99.00 – 118.71	20.20 – 30.49
Deficiency%	-	-	100.0	100.0	0.00	100.0
Bathua *(n=14 samples from 5 sites)*						
Mean	0.73±0.03	0.16±0.02	17.13±2.00	4.17± 0.59	88.77±18.09	37.03±3.36
Range	0.56 - 0.90	0.09 – 0.34	8.72 – 37.79	0.00 – 7.17	32.00 – 208.03	23.56 – 57.22
Deficiency%	-	-	100.0	100.0	33.33	58.33
Oats (n=12 samples from 3 sites)						
Mean	0.34±0.02	0.18±0.02	18.08±1.37	4.70± 0.76	127.22±15.35	43.09±4.81
Range	0.25 - 0.41	0.13 - 0.27	15.50 – 23.26	3.13 6.25	98.68 – 173.36	26.93 – 57.22
Deficiency%	-	-	100.0	100.0	0.00	20.0
Berseem *(n=7 samples from 2 sites)*						
Mean	1.02±0.10	0.25±0.03	17.03±0.82	5.47± 0.78	194.36±50.89	33.66±9.01
Range	0.76- 1.26	0.19-0.32	15.50 – 19.38	3.31–6.25	109.35 – 341.78	13.46 – 47.12
Deficiency%	-	-	100.0	100.0	0.00	42.8

±Standred error of means.

Table 2. Mean concentration of minerals, their range and status in proteinic concentrates, cereals and feed balance

	Ca (g\100g)	P (g\100g)	Zn (µg\g)	Cu (µg\g)	Fe (µg\g)	Mn (µg \g)
Cottonseed (n=38 samples from 7 sites)						
Mean	0.46±0.02	0.86±0.04	46.36±3.01	12.89± 0.84	153.77±10.44	36.35±2.76
Range	0.32 - 0.65	0.54 - 1.35	17.96 - 77.29	6.25 - 25.0	73.13 - 333.33	12.80 - 62.10
Deficiency%	-	-	4.37	21.87	0.00	65.62
Cottonseed-cake (n=34 samples from 8 sites)						
Mean	0.40±0.02	0.86±0.03	45.85±3.68	12.16± 1.29	153.22±10.15	27.92±2.95
Range	0.26 - 0.56	0.60 - 1.12	26.94 - 86.62	6.25 - 18.8	56.91 - 243.90	8.90 - 60.90
Deficiency%	-	-	44.0	12.0	0.00	80.0
Clusterbean seed (n=10 samples from 3 sites)						
Mean	0.32±0.05	0.62±0.08	63.18±8.19	12.50± 1.61	161.80±9.75	24.17±2.68
Range	0.27 – 0.40	0.61 – 0.72	36.94 – 86.20	6.25 – 18.75	130.08 – 198.37	13.60-32.00
Deficiency%	-	-	16.66	16.66	0.00	100.0
Wheat flour/dalia (n=32 samples from 7 sites)						
Mean	0.26±0.01	0.42±0.02	31.66±2.72	10.07± 0.63	142.07±10.97	28.10±1.82
Range	0.18 - 0.38	0.25 - 0.58	12.57 - 86.62	6.25 – 13.2	63.31 - 284.55	16.70 - 51.30
Deficiency%	-	-	69.23	48.15	0.00	88.46
Pearlmillet grain (n=12 samples from 4 sites)						
Mean	1.01±0.05	0.45±0.03	40.46±3.61	11.22± 1.51	169.66±14.41	27.79±2.31
Range	0.78 - 1.19	0.31 – 0.56	26.70- 57.47	6.25 – 18.75	106.70 – 219.57	18.3 – 39.7
Deficiency%	-	-	55.55	55.55	0.00	100.0
Feed pellets (n=10 samples from 4 sites)						
Mean	1.15±0.13	1.34±0.08	35.86±3.36	19.80± 1.92	151.23±14.59	21.45±3.24
Range	0.71 - 1.56	1.04 - 1.64	27.30 - 44.30	12.50 - 26.00	108.20 - 186.99	14.40 – 34.00
Deficiency%	-	-	66.66	0.00	0.00	100.0

± Standred error of means.

Dhigawa or Tosham contained the highest amount (4.17±2.06 ppm). Pearlmillet stover and sorghum stover contained 0.00-6.87 (1.76±1.69) and 0.00 to 6.25 (3.65±0.52) ppm, respectively. All the samples of wheat straw, pearlmillet stover and sorghum stover were deficient in Cu; the value fell much below the 8-10 mg/kg as suggested by NRC (1989). Dry roughages are mostly deficient in Cu because in most circumstances Cu concentration also declined as plants mature (McDowell 1985).

Barring 18% samples of pearlmillet *kadbi* all the samples of dry roughages had Fe more than the level specified by NRC (1989) i.e. 50 ppm. Moreover, many of the samples contained much higher amount of Fe which may hinder the availability of other minerals like Ca, P, Zn and Mn. Higher values of Fe in dry roughages were reported from various parts of Haryana (Yadav *et al.* 1998, Mandal *et al.* 1996). However many samples of soil of Haryana were deficient in Fe (Gupta *et al.* 1981, 1984).

Mn concentration in wheat straw ranged from 10.10 to 57.2 ppm and the average value was 38.25 ppm. Though the average value was very nearer to its requirement (40 ppm) yet 72.2% samples were deficient in Mn. Similarly its concentration in pearlmillet and sorghum stovers ranged from 16.8 to 47.1 and 20.22 to 30.5 ppm. Amongst the samples 81.8% of pearlmillet stover and 100% sorghum stover were deficient in Mn.

The concentration of Ca and P were within the reported range. The level of Ca was slightly higher in sorghum stover in comparison to wheat straw or pearlmillet stover. Overall stovers had higher concentration of Ca than straws, while P concentration was almost similar. If fed alone, dry roughage could maintain 70-100% of maintenance requirement of Ca.

Green roughages

Bathua (*Chenopodium album*) was the major green fodder being fed to the milch animals with straw and stovers. At 2 or 3 sites the farmers were growing berseem and oats. The mineral composition of different green fodders are given in Table 2.

Zn concentration ranged from 8.7 to 37.79, 15.5 to 23.3 and 15.5 to 19.4 ppm on DM basis in *bathua*, oats and berseem, respectively. All these samples of green fodder had below 40 ppm of Zn. However, the values of Zn in berseem collected from Rewari district (Yadav *et al.* 1998) were higher then the present values. The dry roughages, as discussed earlier, and green fodders being fed to the buffaloes were home grown and thus it can be ascertained that Bhiwani district is deficient in Zn. Its deficiency in soil has also be reported by Gupta *et al.* (1981).

Bathua, oats and berseem contained 0-7.17, 3.1-6.3 and 3.1 to 6.3 ppm Cu, respectively. All these samples of green forages were deficient in Cu and could merely supply up to 50% of Cu requirement. Therefore, the area is also deficient in Cu like Zn.

Except 33% samples of *bathua*, all the samples of green fodder had more than 50 ppm on DM of Fe. Very high values of 109.0-342.0 ppm and 99 to 173 ppm were estimated in berseem and oats. Similarly its concentration also reached to 208 ppm in *bathua*.

Mn concentrations were 23.6 to 57.2 ppm in *bathua*, 26.9 to 57.2 ppm in oats and 13.5 to 47.1 ppm in berseem. About 58, 20 and 50% of samples of *bathua*, oats and berseem, respectively, had Mn below the required level of 40 ppm.

Bathua was also a good source of Ca and values (0.73%) were in between the leguminous (1.02%) and non-leguminous (0. 34%). Similarly all the samples contained appreciable quantities of P.

Protein supplements: Cottonseed and cottonseed-cake are the major concentrates offered to animals by majority of the farmers. It was home grown as well as purchased from market. The clusterbean seed is also grown in this district and fed to the animals after boiling at slow heat for overnight. The composition of different minerals in these concentrates is given in Table 3.

Zn concentration ranged from 18 to 77 ppm DM in cottonseed and 27 - 87 ppm in cottonseed-cake. The average values were 46 ppm in both cottonseed and cottonseed-cake. Only 43% samples of cottonseed were deficient in Zn while for cottonseed-cake it was 44%. Zn concentration in cottonseed and cottonseed-cake was almost similar to that of Rewari district (Yadav *et al.* 1998). The cottonseed and cottonseed-cake contained fairly good amount of Cu i.e. 6-25 ppm and 6 -19 ppm, respectively. Though the average values of Cu in different sites of both the ingredients were above 10 ppm, but 22% of cottonseed and 12% of cottonseed-cake samples were deficient in Cu. Similar values of Cu have also been reported from Mohindergarh district (Mandal *et al.* 1996).

All the samples of cottonseed-cake had more than 50 ppm of Fe. The upper values of the range were 244 for cottonseed-cake and 333 ppm for cottonseed.

Though the cottonseed and cottonseed-cake were fairly rich in Zn, Cu and Fe but concentration of Mn was poor. About 66% of cottonseed and 80% cottonseed-cake samples were deficient in Mn. Yadav *et al.* (1998) observed lower value of Mn concentration in these feed ingredients in Rewari district.

Cottonseed and its cake contain fairly good amount of Ca (0.26-0.56% in cottonseed-cake and 0.32 to 0.65% in cottonseed). Both the ingredients are also rich source of P and concentration ranged from 0.54 to 1.35 in cottonseed and 0.60 to 1.12 ppm in cottonseed-cake. While the values reported earlier from Rewari and Mohindergarh district were slightly lower (Yadav *et al.* 1998, Mandal *et al.* 1996).

Clusterbean seed (*Cymopsis tetragonoloba*) is a good source of protein (32% CP). It contained Zn, 36.94 to 86.20 ppm, Cu 6.25-18.75, Fe 130-198 ppm, Mn 13.60-32.00 ppm, Ca 0.27 to 0.40% and P 0.61 to 0.72%. Therefore it is fairly rich in Zn and Cu. However, all samples were deficient in Mn.

Cereals: Wheat and pearlmillet were the major cereals grown in Bhiwani district. However, appreciable quantity of these cereals is also used as animal feed. The compositions of different minerals in wheat and pearlmillet are given in Table 4. Wheat is offered as flour or *dalia* (coarsely ground wheat). It contained fairly good amount of Zn (31.66±2.72 ppm) which ranged from 12.6 to 86.6 ppm. Pearlmillet was richer source of Zn than wheat. It contained 26.72-57.5 ppm Zn. The average value (40.5 ppm) was also similar to the specified limit. The present estimated values were closer to those reported by Yadav *et al.* (1998). Whole cereal grains are relatively rich in total Zn (McDowell 1992) as most of the Zn is present in bran and germ portions. A considerable part (80%) is lost in milling, however, whole grains, either in the form of flour or *dalia* and milling byproducts are generally fed to the animals. Therefore, animals are receiving appreciable quantity of Zn through these cereals.

The concentration of Cu ranged from 6.25 to 13.2 ppm in wheat and 6.25 to 18.75 ppm in pearlmillet. The average values were 9.61 and 9.72 ppm, respectively, and were closer to the required values (10 ppm). However, 48 to 55.5% of the samples of respective grains were deficient in Cu. Cereal grains generally contain 4.8 ppm Cu as reported by Davis and Mertz (1987).

Iron content was also higher like other minerals. The range was 63.3 to 285.0 and 107 and 220 in wheat and pearlmillet, respectively. Most cereal grains generally contain 30-60 ppm Fe and species difference appears to be small (McDowell 1992). Similar phenomenon also existed in the present study, however, the values were much higher than the reported ones.

The concentration of Mn was lower than the required level. The means values were 28.1 and 27.8 in wheat and pearlmillet, respectively. A great variation existed in Mn content (16.70 to 51.30 ppm) for wheat while it was lesser in pearlmillet (18.3 to 39.70 ppm). Generally Mn is concentrated in outer layers of grains (McDowell 1992). Therefore, brans are rich sources of Mn than the whole grain.

Though cereals are deficient in Ca but pearlmillet grain is exception to that. It contained 1.1% Ca and variation was also less (0.78 to 1.2%). Wheat and pearlmillet contained almost similar quantity of P (about 0.40%).

Feed pellets: Feed pellets marketed some agencies are also offered to the animals by some progressive farmers. The composition of different minerals is given in Table 4. The concentrate mixture complete in all respect is pelleted. However, 67 and 100% of feed pellets had Zn and Mn far below the respective specified levels (NRC 1989).

Majority of samples of dry and green fodders (wheat straw, pearlmillet stover, sorghum stover, berseem, oats, *bathua*) and cereal grains in this district were deficient in Zn, Cu and Mn. Cottonseed, cottonseed-cake and clusterbean seed though supplied good amount of Zn and Cu but were deficient in Mn. Fe content of feeds and fodders are quite high, which may affect the utilization of other minerals. Excess Fe reduces

availability of P though the formation of insoluble ferric phosphate (Suttle 1967) and the availability of Zn and Cu also reduces due to excess dietary Fe (Underwood 1977, Buck 1978). The extensive use of Fe appliances in agricultural operations might be the reason of higher Fe concentration in the fodder samples. Reports (McDowell 1992) from tropical regions have also cautioned that forages grown on acid soils are extremely high in Fe, which may aggravate the low Cu status. The excess Fe in feeds and fodders might have also lead to precipitate P and Zn deficiency, besides their poor dietary supply.

Mineral concentration also varied at different sites and also within sites which seems to be natural. Animals are mainly stall fed with limited or no grazing and confined within a limited area. The use of common salt and mineral mixture supplementation was not a common practice except at some organized dairy farms or on recommendation of Veterinary Practitioners under certain diseased condition. This observation on feeding of mineral mixture and even common salt to the cows and buffaloes were consistent to that of Mahindergarh (Mandal *et al.* 1996) and Rewari (Yadav *et al.* 1998) districts of Haryana state. On the basis of feed intake data collected from the farmers, the intake of different minerals was calculated as Ca 54.76±2.06 (19.6-95.3g), P 46.51±2.53 (13.6-76.1g), Zn 29.96±0.60 (22.0-35.7 ppm), Cu 6.47±0.17 (4.2-8.9 ppm), Fe 124.93±2.87 (86.4-168.4 ppm) and Mn 31.26±0.75 (25.1-44.7 ppm) ppm in lactating buffaloes. In heifer the values of intake of different minerals were Ca 25.74±1.37 (16.8-35.0 g), P 8.25±0.59 (4.2-18.0 g), Zn 22.95±0.89 (18.0-33.0 ppm), Cu 3.31±0.32 (0.8-6.0 ppm), Fe 125.3±6.40 (85.8-172.0 ppm) and Mn 36.33±1.40 (23.9-44.4 ppm) ppm. The intake values for Zn, Cu and Mn were far below than the requirements of 40,. 10 and 40 ppm, respectively, as specified by dairy cattle of all ages and physiological functions (NRC 1989). The deficiencies or short supply of minerals also corroborated well with the different diseases/syndromes like anorexia (P and Zn), reproductive disorders (Ca, P, Cu and Zn), milk fever (Ca), retained placenta (Cu and Ca), rough hair coat, dry and scaly skin (Zn) encountered during survey and by taking history of animals from the owner and veterinary surgeons.

Supplementation of Mn (15 to 16 mg), Cu (6 to 8 mg) and Zn (18 to 20 mg) /kg diet would meet the requirement of these minerals in Bhiwani district. Very high yielding buffaloes and cows also need supplementation of limestone or $CaCO_3$ especially during scarcity of green. Supplementation of Fe is not required in mineral mixture to be prepared for this district.

REFERENCES

Aggett P J, Mills C F, Plant J A, Simpson P R, Stevenson A, Dingwall-Fordyce I and Halliday C F. 1988. A study of environmental geochemistry and health in north east Scotland. *Geochemistry and Health. Proceedings of the Second International Symposium.* pp. 81-91 (Ed.) Thronton I. Science Reviews Ltd, Northwood, England.

AOAC. 1984. *Official Methods of Analysis.* 18th edn. Association of Official Analytical Chemists, Washington, D. C.

Buck W B. 1978. *Toxicity of Heavy Materials in the Environment.* (Ed.) Ochne F W. Macel Bekker inc. New York, USA.

Davis G K and Mertz W. 1987. *Trace Elements in Human and Animal Nutrition.* pp. 301. (Ed.) Mertz W. Academic Press, New York.

Gupta V K, Potalia B S and Hans Raj. 1984. Distribution of micronutrients in the soils of Northern region. *Proceedings FAI-NR Seminar* held at Jaipur. pp. 41-70.

Gupta P C, Singh P and Sharda D P. 1981. Distribution of zinc in plant-animal system: A Discussion. *Livestock Advisor* 6: 63-65.

Kanwar J S and Randhawa M S. 1967. *Micro Nutrient Research in Soils and Plants in India- A review.* ICAR, New Delhi.

Kanwar J S and Randhawa M S. 1974. *Micronutrient research in soils and plants in India- A review.* ICAR Tech. Bull. (Agri.) No. **50.**

Mandal A B, Yadav P S, Sunaria K R, Kapoor V and Mann N S. 1996. Mineral status of buffaloes in Mohindergarh district of Haryana. *Indian Journal of Animal Sciences* 66: 849-851

Mc Dowell L R. 1985. *Nutrition of Grazing Ruminants in Warm Climate.* Academic Press New York.

Mc Dowell L R. 1992. *Minerals in Animal and Human Nutrition.* Academic Press New York.

Nayyar V K, Takkar P N, Bansal R L, Singh S P, Kaur N P and Jadana J S. 1990. Micro nutrients in soils and crops of Punjab. *Research Bulletin* PAU, Ludhiana. pp. 146.

Dangarwala R T. 1994.(fide in *Micro nutrient and Sulphur research in Gujarat.* Micro nutrient project (ICAR), Gujrat Agriculture University, Anand. pp. 56.)

NRC. 1989. *Nutrient Requirements of Dairy Cattle.* 6th edn. National Academy of Science, Washington DC

Pathak N N and Verma D N. 1993 *Nutrients Requirements of Buffaloes.* International Book Distributing Agency, Charbag, Lucknow.

Underwood E J. 1977. *Trace Elements in Human and Animal Nutrition.* 4th edn. Academic press, New York, USA.

Yadav P S, Mandal A B, Kapoor V, Sunaria K R and Mann N S. 1998. Mineral status of cows and buffaloes in Rewari district of Haryana. *Indian Journal of Animal Sciences* 68: 1059-61.

Indian Journal of Animal Sciences **73** (10): 1155-1158, October 2003

Limiting amino acids in the bypass protein fraction of some commonly used feedstuffs

K T SAMPATH[1], M CHANDRASEKHARAIAH[2] and A THULASI[3]

National institute of Animal Nutrition and Physiology, Adugodi, Bangalore, Karnataka 560 030 India

Received: 10 Novrmber 2002; Accepted: 25 June 2003

ABSTRACT

Studies were conducted to determine the availability of limiting amino acids (lysine and methionine) in the bypass protein fraction of some commonly used feedstuffs. The feedstuffs, viz. groundnut-cake, cottonseed-cake, maize gluten meal (40%CP), silkworm pupae, bajra grain, broken rice, rice polish and navanae (*Setaria italica*) contained 0.85 and 0.25; 1.65 and 0.34; 1.33 and 0.88; 1.71 and 1.08; 1.48 and 0.11, 0.70 and 0.10, 0.95 and 0.17 and 1.48 and 0.14% lysine and methionine respectively. The above feedstuffs were incubated in nylon bags in the rumen of three fistulated-crossbred steers for 24 hr. The lysine and methionine disappearance from these feedstuffs during 24 hr incubation in the rumen were 76.25 and 65.43; 47.86 and 57.84; 81.92 and 52.65; 26.39 and 26.19; 83.48 and 74.69, 88.48 and 96.98, 71.99 and 78.01 and 76.99 and 74.98% in groundnut-cake, cottonseed-cake, maize gluten-meal (40%CP), silkworm pupae, pearl millet grain, broken rice, rice polish and navanae (*Setaria italica*) respectively. The bypass protein fractions of cottonseed-cake and silkworm pupae were good source of lysine and the bypass proteins fractions of maize gluten-meal (40%CP) and silkworm pupae were good sources of methionine.

Key words: Animal nutrition, Bypass protein, Feedstuffs, Lysine, Methionine

High yielding dairy cows have a large requirement for nitrogen in the form of amino acids. These amino acids are derived from the microbial protein synthesized in the rumen and from the undegraded dietary protein (UDP)/bypass protein. Microbial protein synthesized in the rumen cannot supply all of the protein needs of high producing cows and hence a substantial amount of dietary metabolizable protein must be fed to achieve high milk production (NRC 1985). However, results of the studies in dairy animals fed higher levels of bypass protein have been inconsistent. Some researchers have reported increased milk yields when fed higher levels of bypass protein in the ration (Sampath *et al.* 1997, Kalbande and Thomas 1999, Volden 1999, O'Mara *et al.* 2000, Chaturvedi and Walli 2000), while others have not observed any significant increase in the milk yield. (Christenson *et al.* 1993, Keeri and Amos 1993, Baker *et al.* 1996, Huyler *et al.* 1999). A possible reason for the inconsistent results may be attributed to the differences in the post ruminal supply of amino acids with different bypass protein sources. Methionine and lysine are often reported to be the first limiting or co-limiting amino acids for milk production (Schwab *et al.* 1992, Depeters and Cant 1992, Murphy and O'Mara 1993 Rulquin 1994). Thus feeding of bypass protein sources that supply methionine and lysine post ruminally may result in increased milk production as compared to those which are deficient in these amino acids. However, the information about the methionine and lysine content in the bypass protein fractions of commonly used feedstuffs is lacking. This applies not only to the protein sources but also to the other feedstuffs, which constitutes substantial portion of the concentrate mixture that is fed to dairy animals. The lysine and methionine content of some commonly used protein sources have been reported earlier (Sampath *et al.* 2002). In the present study, the level of lysine and methionine content in the bypass protein fraction of some commonly used feedstuffs along with some more protein sources is reported.

Present address: [1] Principle Scientist, [2,3]Scientist.

MATERIALS AND METHODS

Collection of bypass protein fraction of feeds from rumen

Adult crossbred steers (3) of about 350 kg body weight fitted with large rumen cannula were used to obtain the bypass protein fractions of the feedstuffs selected for the study. Animals were fed concentrate mixture (maize 30%, groundnut-cake 25%, wheat bran 42%, mineral mixture 2% and salt 1%), green grass and ragi straw to meet the nutrient requirement for maintenance (ICAR 1985). Feedstuffs such as groundnut-cake, cottonseed-cake, maize gluten-meal (40%CP), silkworm pupae, pearl millet grain, broken rice, rice polish and navanae (*Setaria italica*) were used for the

study. The feed samples were ground to a particle size of 2-3 mm. The samples were placed in nylon bags (pore size 40 mm) of size 100 mm × 170 mm and suspended in the rumen of fistulated animals for 24 hr, then removed, washed and dried to a constant weight at 60°C. The lysine and methionine content in these rumen-incubated samples (bypass protein fractions) were estimated. Each sample was tested in duplicate for two consecutive days in the rumen of 3 animals. The data were analysed statistically (Snedecor and Cochran 1989).

Amino acid analysis

The lysine and methionine content of feedstuffs before incubation in the rumen and in the bypass protein fractions were determined in a high pressure liquid chromatography (HPLC) using pre-column derivatisation with phenyl isothiocyanate (PITC).

Hydrolysis: Standard hydrolysis procedure (Fountoulakis and Lahm 1998) was followed to obtain the hydrolysate with following modifications using indigenous materials. Narrow neck glass test tubes of 30 ml capacity specially designed for the purpose (to facilitate sealing) were used for hydrolysis of the samples. 0.1g samples were weighed into these tubes. Ten ml of 6N HCl was added and the tubes were purged with nitrogen for 10 sec and sealed by heat using LPG and oxygen simultaneously through a burner. The samples were placed in oven at 110°C for 24 hr. After removal from the oven, the samples were allowed to cool and the hydrolysed samples were concentrated using a flash evaporator.

Derivatisation : The derivatisation process was carried out as described by Albin *et al.* (2000) with minor modification as given below. 5 ml of diluted hydrolysate/standard were pipetted into 1 ml capacity glass test-tubes of 4 mm diameter. The samples in the tubes were derivatised by adding 20 ml of 7: 1: 1: 1 (V/V/V/V) methanol: water: triethylamine: phenyl isothiocyanate and mixed by holding the tubes at a 45° angle and vortex mixed for 20 min. These tubes were then capped and derivatization was allowed to occur for about 35 min at room temperature. The samples were vacuum dried for 4 to 6 hr following derivatization. The samples were then reconstituted in 200 ml of sample diluent. The sample diluent composed of a 95: 5 (V/V) phosphate buffer, (disodium hydrogen phosphate, pH 7.4, adjusted with 10%, phosphoric acid): acetonitrile. The samples were vortexed for 10 min allowed to stand for 15 min and then vortexed again. The injection volume used was 5 ml for each sample.

Analysis: The estimation of amino acids were carried out as per Albin *et al.* (2000), using a Waters HPLC system which consisted of automated gradient controller, two 510 model pumps, a column heater, and a UV detector set at 254 nm. Two eluents were used. Eluent A consisted of sodium acetate trihydrate buffer 940 ml and 60ml of acetonitrile. Eluent B consisted of 60: 40 (V/V) acetonitrile and milli-Q water. Both eluents were vacuum filtered through a 0.20 mm PTFE membrane before use. Amino acid peaks were identified and integrated with Waters Millennium 32 software.

RESULTS AND DISCUSSION

The lysine and methionine content of protein sources before incubation in the rumen is presented in Table 1. Highest amount of lysine (1.71%) was in silk worm pupae followed by that in cottonseed -cake (1.65%), bajra and navanae (1.48%), and maize gluten-meal (1.33%), rice polish (0.95%), and broken rice (0.70%). Highest amount of methionine (1.08%) was observed in silkworm pupae followed by that in maize gluten-meal (0.88%). The methionine content of other feedstuffs ranged from 0.10 to 0.34%. Reddy and Vaidya (1973) have reported the lysine and methionine values of 1.43 and 0.30% for groundnut-cake, 0.85 and 0.49% for cottonseed-cake 1.06% and 1.13% for maize gluten-meal, 0.40 and 0.39% for rice polish, and 0.42 and 0.22% for pearl millet. Gopalan *et al.* (1977) reported the values of 0.25% and 0.16% for lysine and methionine in broken rice. The information on the lysine and methionine content for silkworm pupae and navanae in the literature is limited. Although, the methionine content of the feedstuffs observed in the present study is closer to the values reported earlier, the lysine content is much higher than the reported values except in groundnut-cake. These variations may be attributed to the differences in the varietal strains of the feedstuffs. Lot of new strains of crops with considerable improvements in their nutritive value and pest tolerance capacity have been developed during last 2 decades.

Table 1. Lysine and methionine content of protein sources (per cent on dry-matter basis)

Protein source	Lysine	Methionine
Groundnut-cake	0.85	0.25
Cottonseeed-cake	1.65	0.34
Maize gluten-meal (40%CP)	1.33	0.88
Silk worm pupae	1.71	1.08
Pearl millet grain	1.48	0.11
Broken rice	0.70	0.10
Rice polish	0.95	0.17
Navanae (*Setaria italica*)	1.48	0.14

The percentage of lysine and methionine disappeared from the feedstuffs after 24-hr incubation in the rumen is presented in Table 2. The disappearance of lysine and methionine was highest from broken rice (88.48 and 96.98%) and was lowest from silkworm pupae (26.39 and 26.19%). The lysine disappearance values ranged from 47.86 to 83.48% and that of methionine ranged from 52.65 to 78.01% in other feedstuffs. The disappearance of limiting amino acids was much lower from protein sources as compared to energy sources.

Since the ruminant production is dependent on the supply of specific limiting amino acids (Clark *et al.* 1992 and Mercuen and Titgemeyer 1992), it is of interest to evaluate the relation between feedstuffs characteristics and the profile

Table 2. Disappearance (%) of lysine and methionine from protein sources incubated in the rumen for 24 hr (average of 12 observations)

Protein source	Lysine	Methionine
Groundnut-cake	76.25 ± 1.24	65.43 ± 0.32
Cottonseeed-cake	47.86 ± 1.72	57.84 ± 1.04
Maize gluten-meal (40%CP)	81.92 ± 2.36	52.65 ± 6.61
Silk worm pupae	26.39 ± 1.87	26.19 ± 2.87
Pearl millet grain	83.48 ± 1.02	74.69 ± 6.61
Broken rice	88.48 ± 1.62	96.98 ± 0.42
Rice polish	71.99 ± 0.05	78.01 ± 0.73
Navanae (*Setaria italica*)	76.99 ± 1.53	74.98 ± 5.46

of amino acid flow to the duodenum. The percentage of lysine and methionine left undegraded in the bypass protein fraction of feedstuffs is presented in Table 3. The high amount of lysine was observed in the bypass protein fraction of only cottonseed-cake (0.86%) and silkworm pupae (1.26%). Although the protein sources groundnut-cake and maize gluten-meal and grains like pearl millet and navanae contained high amounts of lysine, much of this amino acid from these feedstuffs is degraded in the rumen. Bypass protein fraction of silkworm pupae, and maize gluten-meal contained high amounts of methionine (0.80 and 0.42% respectively). The methionine in the bypass protein fraction of other feedstuffs was very low (less than 0.2%).

Cottonseed-cake and maize gluten-meal are considered to

Table 3. Lysine and methionine content in the bypass protein fractions of protein sources (per cent on DM basis) (average of 12 observations)

Protein source	Lysine	Methionine
Groundnut-cake	0.20	0.09
Cottonseeed-cake	0.86	0.14
Maize gluten-meal (40%CP)	0.24	0.42
Silk worm pupae	1.26	0.80
Pearl millet grain	0.24	0.03
Broken rice	0.08	0.003
Rice polish	0.27	0.04
Navanae (*Setaria italica*)	0.34	0.04

be good sources of bypass protein (Sampath *et al.* 1999), and are recommended for feeding of high yielding animals (King *et al.* 1988). Our results of the present study further substantiates the high quality of these protein sources as the bypass protein fraction of these feeds are rich in lysine and methionine which are considered to be limiting amino acids for milk production. However, it is essential to protect extensive degradation of limiting amino acids of groundnut cake in the rumen. Amino acids protected from degradation in the rumen have the potential to improve the balance of amino acids available for absorption from the small intestine. Bypass protein fraction of silkworm pupae, which is also a good source of protein, also contained high amounts of lysine and methionine in the bypass protein fraction. The amino acid supply to the ruminants depends on both delivery of the amino acid to the duodenum and subsequent digestibility within the intestine. However, information about the intestinal digestibility of bypass protein fraction of silkworm pupae is limited and needs further investigation. Although, some of the energy sources studied especially, bajra and navanae were rich in limiting amino acids, these amino acids were extensively degraded in the rumen leaving very little quantity for intestinal absorption.

It may be inferred form this study that the bypass protein fractions of cottonseed-cake and silkworm pupae are good source of lysine and the bypass protein fractions of maize gluten-meal and silkworm pupae good sources of methionine. The bypass protein fraction of groundnut-cake is a poor source of amino acid lysine and methionine as these amino acids are degraded in the rumen.

REFERENCES

Albin D M, Jennifer E, Wubben and Gabert, V M. 2000. The influence of hydrochloric acid concentration and measurement method on the determination of amino acid levels in soyabean products. *Animal Feed Science and Technology* **87**: 173-86.

Baker M J, Amos H E, Nelson A, Williams C C and Froetschel M A. (1996). Undegraded protein intake: Effects on milk production and amino acid utilization by cows fed wheat silages. *Canadian Journal of Animal Science* **76** (3): 367-76.

Chaturvedi O H and Walli T K. 2000. Effect of feeding graded levels of bypass protein on nutrient utilization, nutrient partitioning and on performance of lactating crossbred cows. *Indian Journal of Dairy Science* **53** (1): 1-10.

Clark J H, Klusmeyer T H and Cammeron M R. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *Journal of Dairy Science* **75**: 2304 -23.

Christenson R A, Lynch G L, Clark, J H and Yu Y 1993. Influence of amount and degradability of protein on production of milk and milk components by lactating Holstein cows. *Journal of Dairy Science* **76**: 3490 –513.

Depetors E J and Cant J P. 1992. Nutritional factors influencing the nitrogen composition of bovine milk, A review *Journal of Dairy Science* **75**: 2043-70.

Fountoulakis M and Lahm H W. 1998. Hydrolysis and amino acid composition analysis of protein. *Journal of Chromatography* **826**: 109-34.

Gopalan C, Rama Sastri B V and Balasubramaniam S C.1977 *Nutritive value of Indian Feeds.* National Institute of Nutrition. Indian Council of Medical Research, Hyderabad, India.

Huyler M T, Kincaid R L and. Dostal D F. 1999. Metabolic and yield responses of multiparous Holstein cows to prepartum rumen-undegradable protein. *Journal of Dairy Science* **82**: 527-36.

ICAR. 1985. *Nutrient Requirement of Livestock and Poultry.* Indian Council of Agricultural Research, New Delhi.

Kalbande V H and Thomas C T. 1999. Effect of bypass protein on yield and composition of milk in crossbred cows. *Indian Journal*

of Animal Sciences **69** (8): 614 – 16.

Keery C M and Amos H E. 1993. Effect of source and level of undegraded protein on nutrient use and performance of early lactation cows. *Journal of Dairy Science* **76:** 499.

King K J, Huber J T, Sadik M S, Bergen W G and Grant A L. 1988 Influence of protein (CP) sources fed lactating cows on the amino acids (AA) available for digestion and metabolism. *Journal of Dairy Science* **71**: Suppl.1, 159.

Merchen N R and Titgemeyer E C. 1992. Manipulation of amino acid supply to the growing ruminant. *Journal of Animal Science* **70**: 3238-47.

Murphy J J and O'Mara F P. 1993. Nutritional modification of milk protein concentration and its impact on dairy industry. *Livestock Production Science* **35**: 117-34.

O'Mara F P, Murphy J J and Rath M. 2000. The effect of concentrate supplements differing in ruminal protein degradability on milk production and blood metabolism concentrations of diary cows grazing perennial rye grass pasture. *Livestock Production Science* **64** (2-3): 183-91.

NRC. 1985. *Ruminant Nitrogen Usage*. National Research Council, National Academy Press Washington, D, C.

Reddy V R. and Vaidya V S. 1973. Feed composition tables for poultry feeds. *Poultry Gazette* **57** (1): 19-29.

Rulquin H. 1994. Protected lysine and methionine in dairy cow rations. *Feed mix* **2** (4): 24-27.

Sampath K T, Prasad C S, Ramachandra K S, Sundareshan K and Subba Rao A. 1997. Effect of feeding undegradable dietary protein on milk production of crossbred cows. *Indian Journal of Animal Science* **67** (8): 706-08.

Sampath K T, Chandrasekharaiah M, Anandan S and Thulasi A. 1999. Bypass protein for ruminants. *Technical Bulletin No.2*. National Institute of Animal Nutrition and Physiology, Bangalore.

Sampath K T, Chandrasekharaiah M and Thulasi A. 2002 Availability of limiting amino acids in the rumen undegradable protein (UDP) fraction of commonly used protein sources. *Indian Journal of Dairy Science* **55**: 93-96.

Schwab C G, Bozak C K, Whitehouse N L and Mesbab M M A. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. Sequence of lysine and methionine limitation. *Journal of Dairy Science*.**75**: 3503-18.

Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 8th edn. Oxford and IBH Publishing Co., Kolkata.

Volden H. 1999. Effect of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. *Journal of Animal Science* 77: 1905-18.

Indian Journal of Animal Sciences 73 (10): 1159-1162, October 2003

Relationship between average milk production cost and some selected technical and socio-economic factors surrounding dairy herds

A GUNLU[1], C YALCIN[2], H IMIK[3] and K KIRIKCI[4]

University of Selcuk, 42031, Konya-Turkey

Received : 11 August 2002; Accepted : 26 May 2003

ABSTRACT

In this study, data from 77 randomly selected dairy herds surveyed in 1999/2000 were used to determine the relationships between average milk production cost and some selected technical and socio-economic factors surrounding dairy herds. The relationships were quantified by the general linear model equation. The impacts of milk yield, the level of concentrate feed use, number of calf per cow and co-operative membership on the average milk production cost were statistically significant at $P<0.05$. The effects of education level of the producers and the way of provision of roughage to the enterprises on the milk production cost was not significant at $P<0.05$. In spite of the fact that the statistical association between herd size and milk production cost was not statistically significant at $P<0.05$, a statistically significant interaction term ($P<0.05$) between size of farm (HERD SIZE) and main job (FULL-TIME) indicates that only those farmers doing full time farming managed to lower the production cost as they expand the scale of production.

Key words: Cost, General linear model, Milk production, Socio-economic factor

The modern dairy herds operate with a narrow profit margin per kg milk production. An ability of the producer/herd manager to control cost of production is therefore most important factor to distinguish between successful and unsuccessful dairy producers (Mourtis *et al.* 1997). Because of this fact, dairy producers/herd managers need to know about not only factors affecting the cost of milk production, but the direction and magnitude of the relationships as well. Such information helps dairy farmers and their advisors to decide what kind of action to be taken to improve profitability of dairy production.

There is substantial number of papers, which estimated the impacts of some technical (Dhas *et al.* 1990, Ledin and Lema 1996, Zanzad *et al.* 1990), economic (Iype *et al.* 1993, Park 1991, Zanzad *et al.* 1990), and socio-demographic factors (Faruk and Yener 2000, Kairon *et al.* 1995) on milk yield. The results of these studies depict that the signs and magnitudes of the relationships could vary according to the regional and national conditions (Faruk and Yener 2000, Kairon *et al.* 1995, Tripathi and Kunzru 1993).

This study aimed at estimating the relationship between average milk production cost and some selected technical and socio-economic factors surrounding dairy farms in Usak Province, which is important milk production centre in Turkey. The important novelties of this research compared to the above mentioned literature are the use of average milk cost rather than milk yield as a dependent variable, and the examination of possible interactions between independent variables entered the model, apart from the place (Turkey) where no such research was performed before.

Present address: [1] Faculty of Veterinary Medicine, [2]Faculty of Veterinary Medicine, University of Ankara, Dýskapý-06110 Ankara-Turkey.

[3]Faculty of Veterinary Medicine, University of Ataturk, Erzurum -Turkey.

MATERIALS AND METHODS

The data used in the study were collected from 77 dairy herds in Usak Province in Turkey between 1999 and 2000 by conducting an interview survey. A stratified random sampling method was employed for the selection of the herds (Ray 1993).

Cost factors were determined as feed cost, labour cost (including family cost, hired labour cost and herdsmen), cost of veterinary services and drug, repair and maintenance, cost of fuel and electricity, depreciation cost (including building, machinery and livestock depreciation), and miscellaneous costs (change in inventory values). Then, average milk production cost (Turkish Lira-TL/cow/year) was calculated following the below mentioned formula suggested by Acýl (1974):

$$AC = (TC - R)/MY$$

Where,

AC, average milk production cost (Turkish Lira-TL/cow/

year), TC, total costs (TL/cow/year), R, total revenue from by-products (TL/cow/year), and MY, milk yield (kg/cow/year).

A general linear model equation was used to estimate the marginal impact of some selected technical and socio-economic factors surrounding dairy farms in Usak Province on the average milk production cost. The model was:

Cost = f (fulltime, EDU, bred, roughage, coop-member, herd-size, calves, concentrate, MY, I);

where,

Cost, average milk production cost (Turkish Lira-TL/cow/year); full time, does the farmer do full time farming? (1=yes, 2=No); EDU, education level of the farmers (1=primary school, 2=higher than primary school; bred, breed of cows (1=Holstein, 2=Others); roughage, the ways of roughage provision to the farms (1=own production, 2= purchased); coop-member, is the farmer a member of any co-operative related to milk production (1=yes, 2=No); herdsize, number of cows in the farm (head/herd); calves, average number of calves born from each cow (head/cow/year); concentrate; consumption of concentrate feed (kg/cow/year); MY, average milk yield (kg/cow/year); and I, interaction terms between the independent variables.

Amongst the independent variables, fulltime, EDU, roughage and coop-member were entered the model as "factor type variable" and the rest as "continuous variable". The general linear model (GLM) procedure in SPSS statistical software package was used to establish the model. The advantage of using GLM procedure is that it can handle factor type of variables in the model and present both ANOVA tables and co-efficient estimates of the independent variables. GLM firstly corrects the dependent variable according to the effects of co-variates (these were calves, herdsize, concentrate and MY in the equation) then compare statistical significance of the differences between categorical data (fulltime, education, breed, roughage, coop-member).

Possible interactions amongst all independent variables were investigated, but only those found statistically significant at $P<0.05$, were left in the final model.

RESULTS AND DISCUSSION

The average total cost of milk production, and proportion of each cost components in the average total cost in the dairy enterprises are presented in Table 1.

Feed cost is the most important (Table 1) in the total cost (54.2%). It was followed by labour cost (23.4%). These 2 cost components accounted for 77.6% of the total milk production costs. The depression cost, miscellaneous cost and veterinary services and drug cost were negligible compared to 2 above-mentioned cost components. This finding was similar to the findings of Gunlu and Sakarya (2001).

Estimated co-efficient values of the GLM equation, its ANOVA table and estimated average milk cost in some selected independent variable are presented in Tables 2-4.

Table 1. Average total costs and its components in dairy enterprises

	Expenditure (TL)	Expenditure (%)
Total feed cost	1.132.369.029	54.19
Labour	488.414.642	23.37
Veterinary services and drug cost	96.345.012	4.61
Repair and maintenance	15.236.865	0.73
Energy cost	89.284.166	4.27
Depreciation cost	143.332.275	6.86
Miscellaneous	124.667.431	5.97
Total	2.089.649.421	100.00

The impacts of breed, coop-member, calves, concentrate and MY on the dependent variable (cost) and were significant at $P<0.05$ (Table 1). The farmers having Holstein breed cows, greater number of calves (head/cow/year) and higher milk yield (kg/cow/year) had lower milk production cost than their counterparts. On the contrary, co-operative memberships and using high level of concentrate feed (kg/cow/year) were associated with a higher milk production cost.

The associations between the education level of the farmers and the ways of roughage provision to the farm and the milk production cost was not statistically significant ($P<0.05$). The main effect of fulltime and herdsize on the milk production cost were not statistically insignificant ($P<0.05$), but their interaction terms were statistically significant ($P<0.05$).

Insignificant statistical association between milk production cost and co-operative membership may be explained by the facts that the majority of the co-operatives the producers joined were multipurpose agricultural credit and marketing co-operatives, rather than specialised dairy co-operatives. These co-operatives were not successful in providing cheaper inputs and cost of borrowings, and helping the farmers to sell their product at a desired price. This finding shows that the positive impact of co-operative membership

Table 2. The coefficient estimates of the GLM equation

Depend variable	Parameters	Beta	SE	P Value
	Intercept	95804.8	14086.3	0.000
	Fll-time	25318.2	14253.08	0.080
Cost	Education	-4737.9	4476.43	0.294
	Breed	-10062.8	4450.75	0.027
	Roughage	-5905.6	4648.56	0.208
	Coop-member	12317.6	5025.93	0.017
	Calves	-40557.2	10272.97	0.000
	Concentrate	6.4	0.914	0.000
	Herd size	-480.9	773.00	0.536
	MY	-7.7	1.885	0.000
	Full time	- 7005.9	3112.94	0.028

R^2= 0.49.

Table 3. ANOVA table of the GLM equation
(tests of between-subjects effects: dependent variable= average milk production cost)

Source	Type III sum of squares	Df	Mean square	F value	P value
Corrected model	18945964884.823	10	1894596488.482	8.284	.000
Intercept	12590277410.925	1	12590277410,925	55.048	.000
Full-time	721671835.859	1	721671835.859	3.155	.080
Education	255669784.104	1	255669784.104	1.118	.294
Breed	1169140810.720	1	1169140810.720	5.112	.027
Roughage	369129762.414	1	369129762.414	1.614	.208
Coop-member	1373763695.155	1	1373763695.155	6.006	.017
Calves	3564797711.544	1	3564797711.544	15.586	.000
Concentrate	11218378121.280	1	11218378121.280	49.050	.000
Herdsize	1535549027.899	1	1535549027.899	6.714	.012
MY	3782801107.955	1	3782801107.955	16.539	.000
Fulltime * herdsize	1158436894.414	1	1158436894.414	5.065	.028
Error	15095095343.563	66	228713565.812		
Total	451480119489.102	77			
Corrected total	34041060228.386	76			

is directly dependent upon the efficiency of a co-operative organisation fulfilling its stated objectives. Because of these inefficiencies, several studies reported that between 61 to 73% of the modern dairy producers do not believe the usefulness of co-operative organisations in Turkey (Faruk and Yener 2000).

In the literature, the dependent variable was 'milk yield' rather than 'milk production cost'. However, these 2 variables are strongly correlated. Higher milk yield should be reflected as a lower production cost under the "ceterus paribus" assumption. The impacts of co-operative membership on milk yield were reported statistically insignificant by Zanzad *et al.* (1990), but significant by Dhas *et al.* (1990). Different findings related to impact of co-operative membership on milk yield in different countries supported the arguments stated at the forgoing paragraph.

The finding that Holstein breed was associated with a lower milk production cost in this study could be explained by the facts that this breed is adapted to the region better than the other breeds due to government supported Holstein Breeding Projects in the region, and much better production records were being kept under the supervision of the Holstein Breeding Associations. Although average milk yield of the breed is also higher, this is reflected by another independent variable (MY) in the model. Ledine and Lemma (1996) reported statistically insignificant association between breed of cows and milk yield. This contradictory findings can also be explained by the country specific conditions.

Provision of roughage from farmers' own source is one of the important factors to enhance profitability. The farmers who provided the roughage within their own farm sources had lower milk production cost than those purchasing roughage, but the statistical association was not significant ($P<0.05$). This finding may be because the cost of roughage production was closer to the market price of the purchased roughage.

Milk production cost reduced as the calving rate increased. It is obvious that productivity and profitability in dairy enterprises can be enhanced with better fertility management. Provision of replacements from farms own sources is important for both health management and financial management aspects. Mourtis *et al.* (1997) and Park (1991) stressed the positive impacts of providing replacements from farmers' own source on controlling cost of production and obtaining higher profit.

In the intensive dairy farming, cost of concentrate feed is the most important cost component. The level of concentrate use, therefore, closely related to higher milk production cost. The concentrate feed market in Turkey is rather oligopsonic. On the other hand, the dairy farmers are not well organised. High quality concentrate feed is expensive; on the other hand

Table 4. The estimated average milk cost under some selected independent variables.

Independent variable	Levels	Average milk cost (TL/cow/year)	
		Mean	SE
Fulltime	1	76276.8	4777.7
	2	74844.8	2798.2
Education	1	73874.3	2528.6
	2	77188.2	4158.1
Breed	1	71414.5	2643.6
	2	79851.2	3979.3
Rlughage	1	71947.3	2391.0
	2	78378.7	4218.8
Coop-mamber	1	78795.3	2852.3
	2	70622.7	4010.4

cheaper feed has a quality problem. To support dairy farmers, the feed market should be regulated by the Turkish Government for the provision of quality feed at a desired price. If this is not fulfilled, it is quite difficult to increase profitability of Turkish dairy enterprises. The finding reported by Karion *et al.* (1995) in this subject was supported by the findings of this research.

The finding that the higher the farmers education level, the higher the cost of milk production is an unexpected result. However, it was observed during the field survey that the farmers with higher education level tend to employ labour outside the farm, whereas the farmers having lower level of education mainly employed family labour. The farming experience of the farmers with higher education level was also lower than those with lower education level. Iype *et al.* (1993) reported a statistically insignificant association between the level of education and milk yield. However, in some other researches the impact of managerial ability of farmers on milk yield was highly significant (Dhas *et al.* 1990, Tripathi and Kunzru 1993).

In spite of the fact that the main effect of herd size on the milk production cost was not statistically significant ($P<0.05$), a statistically significant interaction term ($P<0.05$) between herd size and full time farming indicates that only those farmers doing full time farming managed to lower the milk production cost as they expand the scale of production.

The adjusted R^2 was 0.49, which means that the independent variables entered in the model explained 49% of variations in the dependent variable.

Low adj. R^2 of the equation does not necessarily imply unreliability in the model estimates. However, the low R^2 indicated that while majority of the above-considered technical and socio-economic factors were important, other elements not considered in the equation accounted for a greater variation in the dependent variable (average milk production cost).

It may be concluded that significant statistical associations between some technical and socio-economic factors surrounding dairy cattle enterprises and their realised milk production cost depicted that there is scope to increase profitability via reducing average total milk production cost by correcting the factors negatively affecting the production cost in dairy cattle breeding enterprises.

ACKNOWLEDGEMENT

Thanks to dairy cattle breeding enterprises owners in Usak province for their helps on data collecting during survey applications.

REFERENCES

Acil F. 1974. *Tarýmsal urun maliyetlerinin hesaplanmasý ve memleketimiz tarýmsal ürün maliyetlerindeki geliþmeler*. pp. 35. A.Ü. Ziraat Fakultesi Yayýnlarý No. 91. Ankara.

Dhas A C, Doornbos M and Nair KN. 1990. Structure of milk production in Tamil Nadu: an analysis of trends and the sources of growth. *CAB Abstracts*. **83**.

Faruk I and Yener M. 2000. Tokat ili merkez ilçesinde ithal sýðýr yetiþtiren tarým iþletmelerinin yapýsý. *Türk-Koop Ekin Dergisi* **12**: 52-55.

Gunlu A and Sakarya E. 2001. Konya sut sigircilik isletmelerinde karlilik ve verimlilik analizleri ile isletmelerin uretim ve pazarlama sorunlari. *Vet. Bil. Derg.* **17**: 97-105.

Iype S, Radhakrishnan J and Raghavan K C. 1993. Influence of socio-economic factors on milk production of crossbred cows in Kerala. *Indian Journal of Animal Sciences* **12**: 1299-1301.

Kairon R S, Singh R V, Harbir Singh and Singh H. 1995. Resource use efficiency and optimum allocation in milk production on small farms in northern Haryana. *Journal of Dairying, Foods-and-Home-Sciences* **3**: 121-30.

Ledin I and Lema A. 1996. Associations between some social, structural and technical factors and the milk production of dairy cows. *Swedish Agriculture Res*earch **26**: 19-30.

Mourtis M C M, Dijkhuizen A A, Huirne R B M and Galligan D T. 1997. Technical and economic models to support heifer management decision: basic concepts. *Journal of Dairy Science* 7: 1406-15.

Park M S. 1991. The rational management improvement of roughage production and dairy farming in Korea. 1. Selection of optimal dairy farming by cropping patterns of forage crop and dairy farming. *Livestock* **33**: 22-30.

Ray W J.1993. *Methods Toward a Science of Behaviour and Experience*. Broke/Cole publishing Co., Pacific Grove. California.

Tripathi H and Kunzru O N. 1993. Differences between the levels of productivity of dairy animals according to psychological attributes of rural women in dairy co-operative. *Journal of Dairying Foods and Home Sciences* **12**: 76-82.

Zanzad A A, Ingole N P and Saigaonkar P B. 1990. Impact of rural dairy co-operatives on development of dairy cattle production in Nagpur district. *Cheiron* **19**: 229-30.

Indian Journal of Animal Sciences 73 (10): 1163-1167, October 2003

Effect of stocking rates on the performance of N'dama cattle in an alley grazing system

J W SMITH[1], A A TAIWO[2], J A AKINLADE[3], A LARBI[4], I O ADEKUNLE[5] and A A BUSARI[6]

Ladoke Akintola University of Technology, P B M 4000, Ogbomoso, Nigeria

Received: 19 August 2002 ; Accepted: 23 May 2003

ABSTRACT

N'dama weaner cattle (12) were used to study the effect of 3 stocking rates (1.0, 1.5 and 2.0 Au/ha) on feed intake and weight gain in an alley farming system comprising hedge rows of *Gliricidia sepium* interplanted with grass *(Panicum maximum)*. The study was carried out in both wet and dry seasons. Factorial treatment combinations, replicated 2-times was used. The effect of season on grass nitrogen content was examined seperately using t-test. N'dama cattle consumed more grass than *G. sepium* in both seasons. Intake of grass was higher in the wet than that in the dry season (mean values: 3.87 ± 0.24 and 1.56 ± 0.48 kg/animal/day respectively). Cattle gained more weight ($P<0.05$) in the wet than that in the dry season (mean values: 245.5±4.4 and 123.8 ± 52g/animal/day respectively). The relationship between weight gain and stocking rate was linear and most pronounced in the west season suggesting that the animal carrying capacity of the land at that season has not yet exceeded. Planting of *G. sepium* in an alley farming system can be used as a technique to conserve and serve as protein supplement to cattle in the dry season when grass forage is limiting factor in quantity and quality.

Key words: Alley grazing, Animal performance, Cattle, Stocking rate

The performance of grazing livestock depends on the quantity and quality of grass forage available to them particularly in the dry season, when forage is in short supply. Stocking rate can be used as a management strategy to optimize animal performance. The relationship between stocking rate and animal performance have been widely studied (Hart 1978, Quigley *et al.* 1984).

The concept of alley farming involves inter-planting grasses within leguminous hedge rows (Kang *et al.* 1990). This integrates livestock into the farming system by making use of hedge row foliage for livestock feeding (Okali and Sumberg 1985). Information exist on the seasonal availability, physical and chemical charateristics of some tree legumes used in alley farming (Larbi *et al.* 1993, Onwuka 1992).

MATERIALS AND METHODS

Leucaena leucocephala and *Gliricidia sepium* are the 2 leguminous browse widely used and studied in alley farming, which provide high protein supplement for livestock feeding (Mani 1984). Supplementation of a basal grass diet with *Leucaena leucocephala* in a stall feeding trial for dairy cows resulted in improved milk yield (Muinga *et al.* 1992). In 1 field study, steers grazing native pasture planted with leucaena gained weight compared to a weight loss in steers without leucaena (Addison *et al.* 1984).

Gliricidia sepium is readily consumed by small ruminants (Ifut 1987). However, the acceptability of the fresh material is low in cattle in west Africa (Kang *et al.* 1990). This is due to its tannin and saponin contents (Onwuka 1992). Nevertheless, the young stems retain much of its leaves and nutrients in the dry season (Carew *et al.* 1981). Thus stocking rate can be used to influence cattle grazing behaviour (El Aich *et al.* 1990) with the aim of ameliorating livestock and biomass performance (Hepworth *et al.* 1991). Therefore, adjustment of stocking rate can be used to influence animal performance and consumption of *G. sepium* by cattle especially in the dry season when grasses are sparse.

Thus, this experiment is designed to study the effect of stocking rates on the feed intake and weight gain of N'dama weaner cattle in an alley farming system comprising grass (*Panicum maximum*) and *Gliricidia sepium* in the wet and dry season.

The study was conducted between December 1992 and August 1993 at the campus of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, which has a

Present address: [1,4,6]International Livestock Research Institute (I L R I) Humid/Subhumid Programme I I T A, P M B 5320 Ibadan, Nigeria.

[2]Institute of Agricultural Research and Training, Obafemi Awolowo University, P M B 5029, Ibadan, Nigeria. [3]Department of Animal Production and Health, Ladoke Akintola University of Technology, P M B 4000, Ogbomoso, Nigeria.

[5]Department of Soil Science and Agricultural Mechanization, University of Agriculture, P M B 2240 Abeokuta, Nigeria.

rainfall of over 1400mm per year and is characterized by tropical rain forest. There are two season with rains between April and October and a dry season from November to March. There is a short dry spell during the rainy season around August/September.

A 1.5 ha *G. sepium/ P. maximum* alley plot was used for the study. Alternative rows of *G.sepium* 4m apart were interplanted with 4 rows of *P. maximum*. The plot was subdivided into 6 paddocks of 0.25ha each N'dama weaner cattle (12), 12- to 18-months-old were randomly allotted to the paddocks. The average initial liveweight of the N'dama cattle was 100 kg. The 3 stocking rates were 1.0, 1.5 and 2.0 animal units (Au/ha). One animal unit is equivalent to 250 kg body weight of animal. Differences in stocking rates were accomplished by varying paddock size. The study was carried out in the dry (Dec-March) and wet (April-Aug) seasons.

Experimental animals were protected against external parasites by dipping in a solution of asuntol and against endoparasites by drenching with 'panacur'. Mineralized salt lick and water were provided throughout the trial.

Animal and herbage measurements

Exclusion cages (4),1m×1m², were randomly placed in each of the paddocks and well anchored into the soil to prevent displacement. At 2 week intervals, 10 open 1×1m² quadrant sampling were taken in each paddocks. Sampling in the exclusion cages was carried out concurrently. Sub-samples from the cuts were randomly taken and oven dried at 60°C for 48 hr for dry matter (DM) determination and ground to pass through an hammer mill of 1 mm diameter for nitrogen analysis.

Dry matter intake was estimated as the difference between the DM yield in the exclusion cage and the DM available in the open quadrant. The cattle were weighed at 2 week intervals. Weight gain of the cattle was calculated as the difference in live weight between 2 successive weight measurements. Slashing of grass and staging of *G.sepium* were 2 techniques used to promote forage utilization when the actual utilization was less than 10% over 2 consecutive samplings. Staged paddock was allowed 3 weeks for recovery before reintroduction of animals. The DM disappearance of *P. maximum* is used as a quality index, was monitored on bi-monthly basis.

RESULTS AND DISCUSSION

The nitrogen content of the herbage (*Panicum maximum*) varied with season (Table 1). The mean dry season value (1.4%) was lower (P<0.01) than that of the wet season. More forage (Fig. 1) was consumed in the wet season than in the dry season (P<0.05) while interaction effect was also not significant (P>0.05).

Grazing N'dama cattle showed a distinct preference for the grass (*P.maximum*) over the browse plant (*G. sepium*) in both seasons. N'dama cattle consumed more grass in the wet

Table 1. Effect of stocking rate on seasonal nitrogen dynamics of *P. maximum* in alley farming systems

Stocking rate (Au/ha)	Dry season	Wet season
1	1.3	2.0
1.5	1.4	2.1
2.0	1.5	2.3
Means	1.4±0.08	2.1±0.12

than in the dry season (P<0.01). Stocking rate had no significant effect on *P.maximum* intake (P>0.5). In the wet season, the intake of grass varied from 3.61 to 4.1 kg DM/ animal/day at 2.0 and 1.0Au/ha respectively. As the stocking rate increases, the grass intake per animal decreases. In the dry season, small quantities of *P. maximum* were consumed compared to the wet season. The values tended to decline as stocking rates increased.

The *Gliricidia sepium* intake was higher (P>0.05) in the

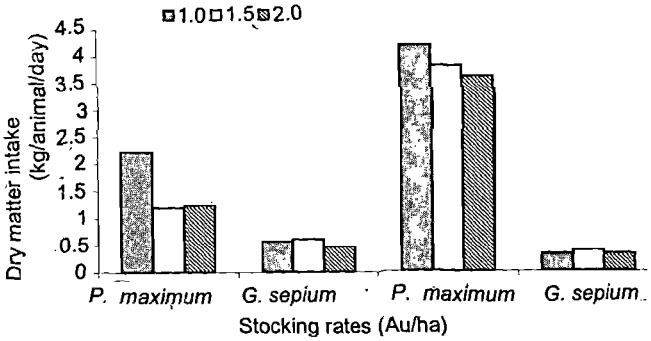


Fig. 1. Effect of stocking rates on dry matter intake of N'dama cattle in the dry and wet seasons in Ibedan, Nigeria.

dry season than in the wet season and not significantly diferent (P>0.05) across the 3 stocking rates. This varied from 0.47kg dry matter intake (DMI) at 2.0 Au/ha to 0.61 kg DMI at 1.5 Au/ha. *Gliricidia sepium* intake tended to decline with increasing stocking rate. The difference in intake at 1.0 and 1.5 Au/ha was not much at 0.57 and 0.61 kg DMI respectively. However, these were higher than the intake at 2.0 Au/ha. The consumption of *G. sepium* in the wet season were almost similar with average intake of 0.34 ± 0.03 kg DM across the 3 stocking rates.

The relationship between *P.maximum* intake (y) and stocking rate (x) was negatively correlated when analysed for wet season (r=-0.560), dry season (r=-0.78*) and both seasons (r=-0.26) combined together.

However, the relationship was only significant (P<0.05) in the dry season. It was defined by the linear regression equation; given under;

$Y = 2.46-0.62x^{(sig)}$; $R^2 = 0.61$ NS- (dry season)

$Y = 3.55-0.53x^{(Ns)}$; $R^2 = 0.31$ NS- (wet season)

$Y = 1.73-0.08x^{(Ns)}$; $R^2 = 0.07$ NS- (wet season)

Weight gain of N'dama cattle were higher (P<0.01) in the wet than that in the dry season for all the stocking rates (Fig.

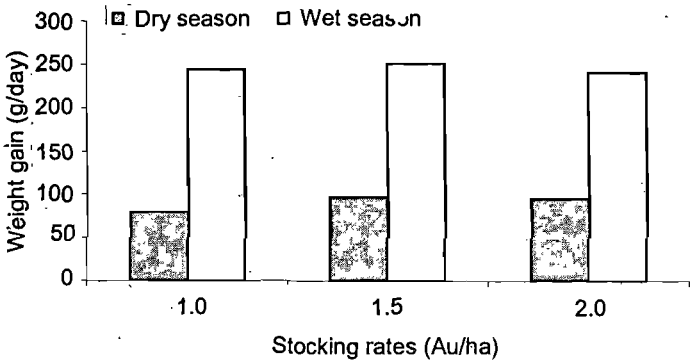


Fig. 2. Effect of stocking rates on weight gain of N'dama cattle grazing *P. maximum* and *G. sepium* pasture.

2). Stocking rate had no effect (P>0.05) on the live weight gain of the cattle. The highest weight gain recorded for wet and dry seasons were 251.3 ± 54.8 and 197 ±62g animal/day, respectively, at stocking rate of 1.5 Au/ha.

The weight of N'dama cattle in the wet season were close to the highest value recorded for stocking rate of 1.5 Au/ha. They had weight gain value of 244.3 ± 47 and 241.9 ± 64g, animal/day respectively.

The relationship between the live weight gain and stocking rate was not significant at the dry season (r=-0.08), wet season (r=0.07) and both seasons (r=-0.05) combined together.

The dry matter degradation characteristics of *P. maximum* did not show any significant difference (P>0.05) at all the stocking rates within each season (Table 2). However, there was seasonal effect on the degradable fractions (b) potential degradability (a+b) and the rate of degradation (c).

Table 2. Degradation characteristics of dry matter of *Panicum maximum* grazed at 1.0, 1.5 and 2.0 Au/ha stocking rates in rumen fistulated cattle in the wet and dry seasons

Season	Stocking rate	Degradation characteristics				
		a	b	A+b	C (% hr)	
Dry	1.0	23.6	37.8	62.8	0.027	
		±2.1	±4.3	±5.2	±0.01	
	1.5	24.4	43.1	68.8	0.031	NS
		±1.9	±7.3	±7.1	±0.01	
	2.0	24.4	44.7	69.0	0.026	
		±1.9	±9.4	±10.6	±0.01	
Dry	1.0	23.9	54.1	78.1	0.043	
		±2.2	±2.8	±3.4	±0.02	
	1.5	25.1	56.3	81.5	0.030	NS
		±1.6	±5.5	±4.8	±0.01	
	2.0	24.1	54.0	78.1	0.041	
		±2.0	±3.0	±3.5	±0.02	
Significance between wet and dry season	NS	S	S	S		

a, Soluble fraction; b, degradable fraction; a+b, extent of degradation; c, rate of degradation; NS, not significant (P>0.05); S, not significant (P<0.05).

The crude protein content of *Panicum maximum* reported in this study (13.13% CP) for wet season, compared favourably well with the value of 12.25 to 13.9% CP recorded for 4-6 week regrowth of *Panicum maximum* in the wet season in another study (Olubajo and Oyenuga 1974). The lower content of nitrogen (crude protein) of *P. maximum* in the dry season can be attributed to the advancing maturity. This results in decline in the CP content and increase in the crude fibre content with advancing forage maturity (Olubajo and Oyenuga 1974).

There was seasonal change in forage intake in this study. Similarly in another study, organic matter intake declined from May (rainy season) to November (early dry season) in Holstein steers grazing wheat grass pasture (Park *et al.* 1994). The succulent nature of the grass in the early wet season, which is rich in digestible carbohydrate (Olubajo 1977) makes more of it to be consumed than in the dry season when it becomes fibrous. Also, its CP content which was higher in the wet than in the dry season makes it more likely to be assumed. The higher consumption of grass recorded in the wet season was also because of its higher digestible nature as depicted by its degradable fraction, extent and rate of degradation (Table 2) than values obtained in the dry season.

A similar result was reported by Park *et al.* (1994). They found that the extent and rate of neutral detergent fibre digestion of wheat grass in May and June was greater than that in September and November. The lower consumption of grass in the dry season might be because of increased grazing pressure.

There was no significant effect of stocking rate on forage intake. This differed from some other reports (Gordon *et al.* 1966, Leaver 1974) where a decrease in herbage production was noticed with increasing stocking rate. Lever (1974) who worked on Friesian calves grazing paddock consisting of pregnant and non-pregnant young female cattle (heifers) used stocking rates of 3.0, 3.5 and 4.0 animal unit per hectare. Whereas, stocking rates of 1.0, 1.5 and 2.0 Au/ha were used in this study. This suggested that the critical/optimal stocking rate at which increasing stocking rate results in reduction in forage intake in the wet season was not observed in this study.

The preference of cattle for grass over *G. sepium* reflected the natural instinct that they are grazer generally, than browser (Rutagwenda *et al.* 1990). The succulent nature of grass in the early rainy season, could also be a contributory factor to their preference. In addition, the levels of tannins and saponins in *G. sepium* could be responsible for its low acceptability and intake (Onwuka 1992) despite its high protein content (Mani 1984).

The low preference of cattle for *G.sepium* was also supported by observations in West Africa where it was observed that *G.sepium* used as live fences were not touched by cattle (Kang *et al.* 1990). However, reports from Sri Lanka showed that *G.sepium* was readily consumed as hand cut

supplement for dairy cows with low weight loss and improved milk production (Chadhokar 1983). Its acceptability in the preceding report may be due to its availability as hand cut fodder. The increased consumption of *G.sepium* at all stocking rates in the dry over the wet season could have been stimulated by the lower quantity and quality of grass available.

The higher weight gain of N'dama cattle in the wet season can be attributed to more feed consumed and the higher quality of the grass forage. This can be inferred from its higher crude protein content and better degradation characteristics in the wet than that in the dry season. The higher potential degradation would make more nutrient available for microbial synthesis in the rumen (Orskov 1989). The higher rate of degradation would induce high rumen evacuation rate thus encouraging more feed intake (Orskov 1989). These factors combine together to make more nutrients available for tissue growth in the lower gut, hence higher weight gain.

Stocking rate did not have significant effect on live weight gain in this study. Mezzadra *et al.* (1992) reported similar results with small sized Aberdeen Angus steers reared at stocking rates, viz. 2.25, 2.87, 3.50 and 4.13 Au/ha (average weight: 174-182 kg). However, they found that with, Limousin (221-247 kg), a large size breed of cattle, the average daily gain declined significantly when the stocking rate was increased from 2.25 to 4.13 Au/ha. Leaver (1974) also reported that stocking rate significantly ($P<0.01$) affect growth rates in calves and heifers particularly from mid and late seasons. The decrease in weight gain of N'dama cattle in the dry season with increasing stocking rates might be due to lower quantity of grass available as a result of increasing grazing pressure and its low quantity. The increase in weight gain with increase in stocking rate from 1.0 to 1.5 Au/ha suggested that the optimum stocking rate, after which the weight gain starts to declined had not been reached. This is supported by a study (Cowlishaw 1968) which reported that the relationship between gain per animal and stocking rate remain linear over a wide range of pasture in the model produced by Peterson *et al.* (1965), it was stated that beyond certain stocking rate, gain per animal declined linearly. The point of decline in weight gain was 2.0 Au/ha in the dry season.

It may be concluded that N'dama cattle when put on pasture containing *P. maximum* and *G.sepium* as hedge row consumed more grass than the browse in both seasons. Intake of grass was higher in the wet than that in the dry season, whereas, gliricidia consumption was higher in dry than that in wet season. Cattle gained more weight in the wet than that in the dry season, and stocking rate had no significant effect on the live weight gain in both seasons.

Intake of *Gliricidia* in an alley farming system can be used to enhance cattle weight gain in the dry season when grass availability is less in quantity and quality.

ACKNOWLEDGEMENTS

The funding provided by ILRI for the execution of the project is acknowledged. The assistance of Dr Bunmi Agbaje (I A R &T, Ibadan) on correlation and regression analysis of data is highly appreciated.

REFERENCES

Addison K B, Cameron D G and Blight G W. 1984. Effects of leueaena and peanut meal supplements fed to steers grazing natural pasture in sub coastal south Queensland. *Tropica Grassland* **18**: 121-30

AOAC. 1990. *Official Methods of Analysis*.15th edn. Association of Official Analytical Chemists. Washington D. C.

Carew R A R, Mba A U and Egbunike G N. 1981. *Nutrition and Systems of Goat Feeding*. pp. 346-51. (Eds). Moran-Fehr, A Bourbouze and M de Sinione. ITOVIE-INRA Tours, France.

Chadhokar P A. 1983. The effect of gliricidia supplemented with dry season forage on milk yield and composition of milking (Netherlands) cows in Sri Lanka. *Tropical Grassland* **17**: 39-41.

Cowlishaw S J. 1968. The carrying capacity of pastures. *Journal of British Grassland Society* **24**: 207-17.

El Aich A, Rittenhouse L R, El Khamkhami S and Ait Mhand T. 1990. Timalh' dit sheep Production and behaviour at three stocking rates in the Moyer Atlas of Morocco. *Journal of Range Management* **43**: 390-96.

Gordon E H, Derbyshire J C, Alexander C W and Me Cloud D E. 1966. Effects of grazing pressure on the performance of dairy cattle on pasture. *Proceedings of 10th International Grassland Congress Helsinki* pp. 470-475.

Hart R H. 1978. Stocking rate theory and its application to rangelands.(Ed) Hyder D N. *Proceedings of the 1st International Rangeland Congress Society Range Manage*, Denver, Colo. pp. 547-50.

Hepworth L W P S, Test R H, Hart J W, Waggoner J R and Smith M A. 1991. Grazing systems, stocking rate and cattle behaviour in Southeastern Wyoming. *Journal of Range Management* **44**: 259-61.

Ifut O J. 1987. 'The nutritive value of *Gliricidia sepium, Panicum maximum* and peels of Manihot spp. fed to west African dwarf goats'. Ph.D. Thesis University of Ibadan, Ibadan, Nigeria.

Kang B T, Reynolds L and ATTA-Krah A N. 1990. Alley farming. *Advance in Agronomy* **43**: 315-59.

Larbi A, Lazier J and Ochang J. 1993. Fodder production and nutritive value of six shrubs on acid soil in southern Ethiopia *Tropical Agriculture (Trinidad)* **70**: 13-16.

Leaver J D. 1974. Rearing of dairy cattle (5) the effect of stocking rating on animal and herbage reduction in a grazing system for calves and heifers. *Animal Production* **18**: 273-84.

Mani R I. 1984. 'Browse selection and intake behaviour of West African dwari sheep and goats'. M.Sc. Thesis University of Ibadan, Ibadan, Nigeria.

Mezzadra C, Escuder I and Migel M C. 1992. Effects of genotye and stocking density on post weaning daily grain and meat production per hectare in cattle. *Animal Production* **55**: 65-72.

Muinga R N, Thorpe W and Topps J H. 1992. Voluntary food intake, liveweight change and lactation performance of crossbred dairy cows given *ad lib. Pennisetum puerperium* (nappier grass var. Bana) supplemented with Leucaena forage in the lowland semi-humid tropics. *Animal Production* **55**: 331-37.

Okali C and Sumberg J E. 1985. Sheep and goats, men and women: Household relations and small ruminant development in

southwest Nigeria. *Agroforestry System* **18**: 39-59.

Olubajo F O. 1977. The nutritive value of hay from four grass species cut at three stages of growth at Ibadan. *Nigerian Journal of Animal Production* **4**: 133-57.

Olubajo F O and Oyenuga V A. 1974. The yield, intake animal production of four tropical grass species grown at Ibadan. *Nigerian Journal of Animal Production* **1**: 217-24.

Onwuka C F I. 1992. Tannin and saponin contents of some tropical browse species fed to goats. *Tropical Agriculture, (Trinidad)* **69**: 176-80.

Orskov R R. 1989. Recent advances in evaluation of roughage as feeds for ruminants. *Recent Advances in Animal Nutrition* (Ed) D J Farrell. University of New England Printery, Armidale Australia.

Orskov E R, Hovell Deb F D and Mould F. 1980. The use of the nylon bags techniques for the evaluation of feed stuffs. *Tropical Animal Production* **5**: 195-213.

Park K K, Krysl L J, McCracken B A, Judkins M B and Holcombe D W. 1994. Steers grazing intermediate wheat grass at various stages of maturity: Effects on nutrient quality, forage intake, digesta kinetics, ruminal fermentation and serum hormones and metabolites. *Journal of Animal Science* **72**: 478-86.

Petersen R G, Lucas H L and Mott G O. 1965. Relationship between rate of stocking and per animal and per acre performance on pasture. *Agronomy Journal* **57**: 27-30.

Quigley T M, Skovlin J M and Workman J P. 1984. An economic analysis of two systems and three levels of grazing on ponderosa pine bunch grass range. *Journal of Range Management* **37**: 309-12.

Rutagwenda T, Lechner-Doll M, Schwartz H J, Schultka W and Von Engelhardt W. 1990. Dietary preference and degradability of forage on an semiarid Thornbush savannah by indigenous ruminants, camels, and donkeys. *Animal Feed Science Technology* **31**: 179-92.

Steel R G D and Torrie J G. 1980. *Principles and Procedures of Statistics*. 2nd edn. McGraw-Hill Books Co., Inc. New York.

Indian Journal of Animal Sciences 73 (10): 1168-1173, October 2003

Growth and density scenario of livestock and poultry in Haryana*

K ELUMALAI[1] and U K PANDEY[2]

CCS Haryana Agricultural University, Hisar, Haryana 125 004 India

Received : 15 October 2002; Accepted : 20 June 2003

ABSTRACT

The present study analysed growth and density based on district-wise livestock census data across regions and state. Indigenous cattle both in milk and dry had declined across regions and state, while crossbred cattle increased over time. She-buffaloes including their young stocks also showed positive growth rates. The positive growth rates of not-calved indigenous and crossbred cattle and buffaloes pose threat to the dairy sector in the state. The population of bullocks in the state recorded negative growth rates, but he-buffaloes registered the annual growth rates of 6.68, 7.20 and 15.22%, during 1972-82, 1982-92 and 1992-97 respectively. Camels in the western region only had 5.95% annual growth rates in 1982-92 inter-census periods. The small ruminants, pigs and poultry also observed positive growth rates across regions and state in all inter-census periods under study. The stocking rates / ha of cropped area for milch buffaloes, indigenous and crossbred milch cattle in the state ranged between 31 and 45, 9 as well as 12 and 2 and 6 respectively. Likewise the stocking rates 1000 persons for milch buffaloes, indigenous and crossbred milch cattle in the state ranged between 126 and 155, 31 and 48 as well as 7 and 9 respectively. Moreover, indigenous bullocks had the highest density both in terms of animal-land and animal-man ratios, followed by he-buffaloes, camels and crossbred bullocks in order across regions and state. Similarly, small ruminants, pigs and poultry also had high densities, both in terms of animal-land and animal-man ratios, across regions and state.

Key words: Animal-man ratio, Animal-land ratio, Compound growth rate, Livestock population, Stocking rate

The livestock sector has emerged as an important segment of an expanding and diversified agricultural sector in the Indian economy. The contribution of this sector is estimated to be about 25% of the total value of agricultural sector (Anonymous 1999). It is the principal source of draught power in rural areas and provides milk, meat, eggs, wool, hides and skins, manure and fuel. Livestock and poultry also play a critical role in agricultural intensification process. Despite it's importance the livestock sector had not received much research attention as the crop sector. Therefore, the present study aims at analysing the species wise growth pattern and density of livestock population across regions and state.

MATERIALS AND METHODS

This study was based on only 12 districts of Haryana state because of availability of time series data for those districts. These districts were grouped into 2 regions, viz. eastern and western regions. The eastern region included the districts of Ambala, Karnal, Kurukshetra, Sonepat, Rohtak and Jind, while the western region comprised Hisar, Sirsa, Bhiwani, Mahendragarh, Gurgaon and Faridabad districts. The secondary data were collected from various published sources such as Livestock Censuses, Statistical and Animal Husbandry Abstracts of Haryana, etc. The district-wise livestock population data were collected for the various census periods such as 1972, 1977, 1982, 1988 and 1992 and was also projected for 1997 based on the compound growth rates of 1982-92. Later on, the livestock population for these census years of corresponding districts were added to generate the species-wise livestock data for eastern and western regions as well as state.

To compute the district-wise and species-wise compound growth rates, the period was divided into 1972-82, 1982-92 and 1992-97. Infact, the compound growth rates of bovines, draught animals, small ruminants, pigs and poultry between the 2 inter-census periods were calculated and also projected for the year 1997 by using the following formula:

$$\frac{P_t}{P_o} = \left(1 + \frac{r}{100}\right)$$

Where, P_t, bovines/small ruminants/draught animals/ poultry birds/pigs population in the tth period; P_o, bovines/ small ruminates/draught animals/poultry birds/pigs population in the base period; r, compound growth rate; and t, time in

This is a part of first author's approved M.Sc. Thesis.

Present address: [1]Ph.D. Scholar, Jawaharlal Nehru University, New Delhi, [2]Professor (Livestock Economics), Department of Livestock Production and Management.

years.

The density analysis of livestock and poultry was extended overtime and space for both in terms of animal-land ratio and animal-man ratio to capture competition for scarce feed and fodder resources across regions and state.

RESULTS AND DISCUSSION

Compound growth rates (CGRs) of bovines, draught animals, small ruminants, pigs and poultry

The indigenous cattle both in milk and dry except for the dry cattle of western region in 1992-97 had declined across regions and state during 1972-82, 1982-92 and 1992-97 inter-census periods (Table 1). Furthermore, the dry cattle population had declined at much faster rate as compared to those in milk. It implies that the indigenous cattle population had steadily decreased over time and space in the state. Such a declining trend in these animals could be attributed to the decline in total indigenous adult female cattle population on the one hand, while the low adoption of proper feeding and management practices by the dairy farmers on the other. These findings are in conformity with those reported by Pandey (1995, 2000). But not-calved indigenous cattle had increased across regions and state in 1972-82 and 1982-92 inter census periods, with a relatively much faster rate in later period.

The population of crossbred cattle in milk, dry and not-calved, had increased during 1982-92 and 1992-97 inter-census periods across regions and state because of their high milk production potential of 3000 litres / lactation. In crossbred dry cattle population across regions and state, the positive growth rates seems to be due to the increasing number of crossbred cattle population. Moreover, the relatively much faster growth trends of crossbred cattle in western region as compared to eastern region signify their importance due to the awareness amongst dairy farmers. The positive growth trends of not-calved crossbred cattle across regions and state pose serious threat to the dairy sector of the state. On the whole, it is apparent that the crossbred cattle were reared for meeting the milk production needs while those of indigenous cattle for draught bullocks across regions and state.

Cattle youngstocks below 3 years, both males and females during 1972-82 and 1992-97 in eastern region, recorded the respective annual growth rates of 0.94% and 1.63% as 4.57% and 8.67%, but declined at the annual rate of 0.20 and 1.67% in 1982-92 (Table 1). Similarly, cattle youngstocks below 3 years, both the males and females in western region and state registered the positive growth rates during all inter- census periods, except for male youngstock in western region during 1972-82 and female youngstock in Haryana during 1982-92. It implies that female young stocks were reared for reproduction purposes and male young stocks for draught bullocks across regions and state.

Buffaloes both in milk and dry including their youngstocks below 3 years had increased across regions and state in all the inter-census periods, except for buffaloes both in milk and dry in eastern region only and in milk in Haryana during 1992-97 (Table 1). It implies that buffaloes were considered as a main milch animal across regions and state probably because of their fat rich milk yield. Moreover, both in milk and dry buffaloes population in 1992-97 inter-census periods declined due to the sale of more number of buffaloes in the current period in comparison to the base period by the farmers to supplement their household income. The positive growth rates of buffaloes' youngstocks clearly revealed the pattern of rearing them for reproduction purposes. Likewise, the

Table 1. Compound growth rates (CGRs) of bovines across regions in Haryana state (% / annum)

Particulars	Eastern region			Western region			Haryana		
	1972-82	1982-97	1992-97	1972-82	1982-97	1992-97	1972-82	1982-97	1992-97
Cattle indigenous									
In milk	-0.27	-1.58	-1.00	-0.88	-0.36	-2.86	-0.57	-0.97	-1.96
Dry	-2.81	-4.07	-7.01	-3.12	-3.76	0.64	-2.95	-4.00	-3.18
Not calved	0.48	4.39	-2.69	0.60	5.07	-1.19	0.53	4.68	-2.02
Crossbred									
In milk	-	5.42	9.53	-	7.95	13.70	-	6.12	10.86
Dry	-	2.04	12.41	-	8.95	34.45	-	3.45	19.98
Not calved	-	10.04	15.52	-	20.68	37.88	-	11.75	22.43
Young stock (below 3 years)									
Male	0.94	-0.20	4.57	-0.83	0.33	2.12	0.20	0.01	3.60
Female	1.63	-1.67	8.67	0.49	0.36	3.54	1.10	-0.73	6.27
Buffaloes									
In milk	2.58	3.17	-1.20	2.52	4.43	0.85	2.56	3.75	-0.21
Dry	0.30	1.97	-0.49	1.20	1.40	2.45	0.65	1.74	0.67
Not calved	11.33	2.25	7.70	9.41	5.47	1.37	10.58	3.55	5.06
Young stock (below 3 years)									
Male	3.27	2.08	3.13	2.64	3.02	7.88	3.02	2.47	5.22
Female	3.76	0.20	1.26	2.74	1.99	3.22	3.30	1.02	2.87

positive growth rates of buffaloes' male youngstocks reflect the replacement rate of these animals for draught purposes across regions and state. These animals compete for the scarce feed and fodder resources across regions and state. Indeed, the increasing trend in buffaloes population including their youngstock might be due to their better adaptability to the local conditions.

Draught animals, small ruminants, pigs and poultry

Compound growth rates of draught animals, small ruminants, pigs and poultry across regions and state are given in Table 2. The bullocks population had declined across regions and state during the study. These declining trends in bullock reflect the replacement of bullocks by tractor by the farmers. Contrarily, he-buffaloes had observed an increasing trend across regions and state in all the periods, except for western region in 1982-92. It could be attributed to the more number of male populations and use of these animals for transport purpose in the state. As such, they compete for the scarce feed and fodder resources across regions and state thereby drawing the attention of policy makers. The camel population declined across regions and state in 1972-82, while the increasing trend was visible during 1982-92. Again, except for eastern region, the camel population declined in western region and state during 1992-97 inter- census periods. It implies that, by and large, camels were replaced across regions and state overtime, which may be due to the modernization of Haryana agriculture.

The sheep population in eastern region increased at the annual rates of 5.02, 4.18 and 3.71%, while those of goats 1.31, 4.65 and 1.58% per annum during 1972-82, 1982-92 and 1992-97, respectively. Similarly, the sheep population in western region increased at the rate of 5.22, 2.66 and 4.79% in 1972-82, 1982-92 and 1992-97, while those of goats 2.85%, and 2.03% per annum in 1972-82 and 1982-92, respectively, while declined marginally in 1992-97. On the whole, the sheep population increased at the annual rates of 5.14, 3.25 and 4.36% in 1972-82, 1982-92 and 1992-97, while those of goats 2.42%, and 2.78% per annum in 1972-82 and 1982-92 inter-census periods, respectively, and marginally declined in 1992-97 (Table 2). This implies that the rearing of small ruminants drew the attention of small and marginal farmers across regions and state probably due to poverty and/or lack of adequate capital assets with them.

The pig population had increased at the annual rates of 5.62, 6.03 and 7.94% in eastern region, while 5.98, 10.78 and 0.74% per annum in western region during 1972-82, 1982-92 and 1992-97 respectively. On the whole, the pig population in the state had increased at the annual rates of 5.72, 7.55 and 5.47% during 1972-82, 1982-92 and 1992-97 inter-census periods respectively (Table 2). It implies that the pig population could also draw the attention of weaker sections in the state, more particularly because of acceptability of these animals by them. Yet, it calls the attention of planners and policy makers to design suitable strategies for promoting the scientific rearing of these animals in the state.

The poultry population had increased at the annual rates of 0.46, 24.20 and 3.33% in eastern region, while 14.62, 6.44 and 0.17% per annum in western region during 1972-82, 1982-92 and 1992-97 inter-census periods respectively. On the whole, the poultry population in the state had increased at the annual rates of 3.89, 15.06 and 2.39% during 1972-82, 1982-92 and 1992-97 inter- census periods respectively. It implies that the rearing of poultry birds could also occupy the place in to the "diversification basket" across regions and state by the people probably due to regular and greater incomes. But it calls the attention of scientists to evolve modern technologies and promote their dissemination while plans and policy makers to frame suitable strategies for promoting its adoption across regions and state.

Table 2. Compound growth rates of draught animals, small ruminants, pigs and poultry across regions in Haryana (% / annum)

Particulars	Eastern region			Western region			Haryana		
	1972-82	1982-92	1992-97	1972-82	1982-92	1992-97	1972-82	1982-92	1992-97
Draught animals									
Bullocks	-2.77	-3.68	-6.30	-2.04	-3.15	-3.38	-2.94	-3.46	-4.90
He-buffaloes	6.39	8.30	16.09	8.31	-0.80	1.74	6.68	7.20	15.22
Camels	-9.75	3.21	2.26	-8.63	6.19	-8.35	-8.74	5.95	-7.46
Small ruminants									
Sheep	5.02	4.18	3.71	5.22	2.66	4.79	5.14	3.25	4.36
Goat	1.31	4.65	1.58	2.85	2.03	-0.84	2.42	2.78	-0.05
Others									
Pigs	5.62	6.03	7.94	5.98	10.78	0.74	5.72	7.55	5.47
Poultry	0.46	24.20	3.33	14.62	6.44	0.17	3.89	15.06	2.39

Density of livestock and poultry

Milch animals and youngstocks : Density of milch animals including their youngstocks across regions and state are given in Table 3. In eastern region, the stocking rates for indigenous milch cattle, crossbred milch cattle and milch buffaloes / ha

Table 3. Density of milch animals and young stock across regions in Haryana

Particulars	Milch animals			Young stock		
	Indigenous cattle	Crossbred cattle	Buffaloes	Indigenous cattle	Crossbred cattle	Buffaloes
Animal- land ratio (per hectare of cropped area)						
Eastern Region						
1982	13	3	38	18	1	37
1988	12	2	41	15	2	32
1992	10	4	45	15	3	39
1997*	9	8	50	15	5	38
Western region						
1982	10	2	31	15	1	30
1988	8	2	33	13	1	27
1992	9	3	37	13	2	32
1997*	9	6	45	13	3	34
Haryana						
1982	12	2	31	15	1	30
1988	10	2	33	13	1	27
1992	9	3	37	13	2	32
1997*	9	6	45	13	3	34
Animal-man ratio (per 1000 persons)						
Eastern region						
1982	48	10	134	65	4	134
1988	47	9	163	60	6	125
1992	32	14	145	50	9	125
1997*	27	24	145	43	16	110
Western region						
1982	49	3	117	58	2	115
1988	45	5	136	61	3	122
1992	35	6	131	44	3	110
1997*	35	14	158	44	6	121
Haryana						
1982	48	7	126	62	3	125
1988	46	7	155	60	4	124
1992	34	10	137	47	6	117
1997*	31	19	152	43	10	116

*Projected population as per compound growth rate of 1982-92 inter-census period by formula Pt/ Po = (1+r/100), where, Pt and Po = population in the t^{th} and base period respectively.

of cropped area ranged between 9 and 13, 2 and 8 as well as 38 and 50 during 1982, 1988, 1992 and 1997 respectively. Furthermore, the stocking rates of their youngstock / ha of cropped area, for indigenous, crossbred cattle and buffaloes raged between 15 and 18, 1 and 5 as well as 32 and 39 respectively.

In the western region, the stocking rates for indigenous cattle, crossbred cattle and milch buffaloes/ha of cropped area were 8 and 10, 2 and 6 and, 31 and 45, respectively, during the study. The stocking rates of youngstock for indigenous, crossbred cattle and buffaloes, however, ranged between 13 and 15, 1 and 3 as well as 27 and 34 respectively.

On the whole, the stocking rates/ha of cropped area for milch buffaloes, indigenous and crossbred milch cattle in the state ranged between 31 and 45, 9 and 12 as well as 2 and 6 respectively. Likewise, the stocking rates / ha of cropped area for indigenous and crossbred cattle and buffaloes youngstocks were between 13 and 15, 1 and 3 as well as 27 and 34 respectively.

The density of milch animals including their youngstock was worked out on the basis of per 1000 persons (Table 3). The stocking rates of indigenous milch cattle in eastern region ranged between 27 and 48, in western region 35 and 49 and for state 31 and 48 / 1000 persons. Likewise, the stocking rate for crossbred milch cattle in eastern region ranged between 9 and 24, western region 3 and 14, and state 7 and 19 respectively. For milch buffaloes, the stocking rates per 1000 persons in eastern, western regions and state, however, ranged between 134 and 163, 117 and 158 as well as 126 and 155 respectively.

For the indigenous cattle youngstock, the stocking rates per 1000 persons in eastern region raged between 43 and 65, in western region 44 and 61 and in state 43 and 62 respectively. Likewise, for crossbred cattle younstocks the stocking rates

per 1000 persons in eastern region ranged between 4 and 16, in western region 2 and 6 and in Haryana 3 and 10. However, buffaloes youngstock in eastern region had density per 1000 persons in a range of 110 and 134, in western region 110 and 122 and, state 116 and 125 respectively (Table 3).

Thus, the density of milch animals including their yougstocks, both in terms of animal-land and animal-man ratios, was the highest for buffaloes followed by indigenous cattle and crossbred cattle across regions and state respectively.

Table 4. Density of draught animals across regions in Haryana

Particulars	Eastern region	Western region	Haryana
Animal-land ratio (per hectare of cropped area)			
Indigenous bullocks			
1982	15	10	13
1988	12	8	10
1992	10	7	8
1997*	7	7	7
Crossbred bullocks			
1982	2	-	1
1988	1	-	1
1992	1	-	1
1997*	3	1	2
He-buffaloes			
1982	4	1	2
1988	5	1	3
1992	7	1	3
1997*	10	1	4
Camels			
1982	-	2	1
1988	-	1	1
1992	-	3	2
1997*	-	4	2
Animal-man ratio (per 1000 persons)			
Indigenous bullocks			
1982	55	48	52
1988	48	44	46
1992	31	28	29
1997*	25	25	25
Crossbred bullocks			
1982	8	1	5
1988	3	1	3
1992	4	1	3
1997*	9	2	5
He-buffaloes			
1982	13	3	8
1988	20	3	12
1992	21	2	11
1997*	28	4	15
Camels			
1982	1	8	4
1988	-	5	3
1992	1	11	6
1997*	1	14	8

*Projected population as per compound growth rate of 1982-92 inter-census periods by formula: Pt/ Po = (1+r/100), where, Pt and Po= population in the tth and base period respectively.

It implies that buffaloes were considered, as the main milch animal while indigenous cattle seems to be reared for meeting out the needs of draught power across regions and state. The crossbred cattle as a milch animal seems to be relatively more popular in eastern region as compared to western region. Youngstocks of these species were also reared across regions for the replacement of herd and/or for the sale of youngstocks to supplement the household incomes.

Draught animals; small ruminants, pigs and poultry : The density of draught animals both in terms of animal-land and animal-man ratios are presented in Table 4. The stocking rates of indigenous bullocks/ha of cropped area in eastern, western regions and Haryana ranged between 7 and 15, 7 and 10 as well as 7 and 13, respectively, during 1982 and 1997. The stocking rates of crossbred bullocks / ha of cropped area ranged between 1 and 3 as well as 1 and 2 in eastern region and Haryana, receptively, during the study. Moreover, he- buffaloes / ha of cropped area, densed between 4 and 10, 2 and 4 in eastern region and Haryana respectively. The density of camels, however, in western region and Haryana had ranged between 1 and 4 as well as 1 and 2, respectively.

The density for indigenous bullocks per 1000 persons ranged between 25 and 55 in eastern region, 25 and 48 in western region and 25 and 52 in state, during the study. For crossbred bullocks, eastern region had relatively high stocking rates ranging between 3 and 9 as compared to western region 1 and 2 while for state being 3 and 5 during periods under study. He-buffaloes in eastern region also had high stocking rates per 1000 persons (13 and 28) as compared to western region (2 and 4), while for state ranged between 8 and 15. Camels had high stocking rates in western region ranging between 5 and 14 as compared to eastern region only one and for state being between 3 and 8 (Table 4).

Thus, indigenous bullocks had the highest density, both in terms of animal-land and animal-man ratios, followed by he-buffaloes, camels and crossbred bullocks in order across regions and state respectively. Hence, indigenous bullocks still seem to be the main stay of farm power across regions and state.

The data about density of small ruminants, pigs and poultry across regions and state both in terms of animal-land and animal- man ratios are shown in Table 5. The western region had relatively high stocking rates both for sheep and goats as compared to eastern region during the periods under study. Indeed, the stocking rates / ha of cropped area for sheep and goats in eastern region ranged between 10 and 23 and 6 and 14, while in western region it ranged between 16 and 25 and 16 and 21 respectively. For state, it ranged between 13 and 24 as well as 11 and 18 respectively. Likewise, the density of sheep and goats per 1000 persons in eastern region ranged between 35 and 67 as well as 23 and 39 while in western region between 77 and 96 as well as 71 and 81 during the study, respectively, thereby indicating the importance of these species. On the whole, density of sheep per 1000 persons in

Table 5. Density of small ruminants, pigs and poultry across regions in Haryana

Particulars	Eastern region	Western region	Haryana
Animal-land ratio (per hectare of cropped area)			
Sheep			
1982	10	16	13
1988	13	17	15
1992	17	20	19
1997*	23	25	24
Goats			
1982	6	16	12
1988	7	15	11
1992	10	17	14
1997*	14	21	18
Pigs			
1982	7	3	5
1988	9	3	6
1992	13	7	9
1997*	17	14	15
Poultry			
1982	26	49	38
1988	95	74	89
1992	252	85	152
1997*	870	130	422
Animal-man ratio (per 1000 persons)			
Sheep			
1982	35	77	55
1988	51	91	69
1992	54	83	69
1997*	67	96	83
Goats			
1982	23	76	47
1988	28	81	52
1992	32	71	53
1997*	39	79	60
Pigs			
1982	26	12	19
1988	37	14	26
1992	40	26	33
1997*	51	52	51
Poultry			
1982	92	230	155
1988	420	404	413
1992	812	345	561
1997*	2525	493	1432

*Projected population as per compound growth rate of 1982-92 inter-census period by formula: Pt/Po = (1+r/100), where, Pt and Po= population in the tth and base period respectively.

the state ranged between 55 and 83 while that of goats ranged between 47 and 60 during the study. Thus, small ruminants had quite high densities across regions and state both in terms of animal-man and animal-land ratios (Table 5).

The stocking rates / ha of cropped area for pigs in eastern, western regions and state ranged between 7 and 17. 3 and 14 as well as 5 and 15 respectively. Similarly, the stocking rates /1000 persons were relatively greater in eastern region (ranged between 26 and 51) as compared to western region (12 and 52) during the study. On the whole, the stocking rates for pigs per 1000 persons in the state ranged between 19 and 51. Thus, pigs were relatively more densed in eastern region as compared to western region both in terms of animal-land and animal-man ratios.

The stocking rates of poultry / ha of cropped area for eastern and western regions as well as state ranged between 26 and 870, 49 and 130 as well as 38 and 422 respectively. Similarly, the density for poultry per 1000 persons were relatively greater in eastern region (ranged between 92 and 2525) as compared to western region (230 and 493) during the study. On the whole, the stocking rates for poultry per 1000 persons in the state ranged between 155 and 1432 during the study (Table 5). Thus, poultry rearing was relatively more popular in eastern region as compared to western region, both in terms of animal-land and animal-man ratios.

On the whole, milch buffaloes and crossbred were considered by the farmers across regions and state as major milch animals. Indigenous cattle were reared for the want of hardy bullocks and their milk is being considered as byproducts (Pandey *et al.* 1983). Undoubtedly, he-buffaloes had increasing trends across regions and state due to their use in transport, yet camels and bullocks had observed a declining trend on account of modernization of Haryana agriculture. Small ruminants, pigs and poultry birds were also reared across regions and state, thereby occupying a place into the " diversification basket". The densities of livestock and poultry birds improved over time and space, both in terms of animal-man and animal-land ratios, which implies greater competition for the scarce feed and fodder resources in the state.

REFERENCES

Anonymous.1999. *Economic Survey* 1998-99.Ministry of Finance, Economic Division, Government of India, New Delhi.

Anonymous. 1999. *Statistical Abstracts of Haryana* (various issues). Economics and Statistical Organisation Planning Department, Government of Haryana.

Anonymous. 1999. *Statistical Abstracts of Animal Husbandry Haryana* (various issues), Director Animal Husbandry, Haryana, Chandigarh.

Pandey U K, Suhag K S and Hashija R C. 1983.Some aspects of bovine economy in Haryana. *Indian Journal of Agricultural Economics* **53**(3): 233-37.

Pandey U K. 1995.The livestock economy of India: A profile. *Indian Journal of Agricultural Economics* **50**(3): 246-82.

Pandey U K. 2000. Livestock in Household Economy. *Agricultural Situation of India;* 447-86.

Indian Journal of Animal Sciences 73 (10): 1174-1179, October 2003

Modelling for growth pattern in crossbred cattle

RAMESH KOLLURU[1], P S RANA[2] and AMRIT KUMAR PAUL[3]

Indian Agricultural Statistics Research Institute, Library Avenue, New Delhi 110 012 India

Received: 30 August 2002 ; Accepted: 17 June 2003

ABSTRACT

The research on cattle growth is one of the important studies in the animal sciences. Although methods are available in literature for the analysis of growth data, no universal method is recommended. In the present study data on body weight was taken from birth to an age up to 27 months on 25 crossbred cattle. As the scatter plots revealed a sigmoid shape and also that the growth of animals, in general follow a non-linear distribution, 5 non-linear models were used to model weight-age data for the animals. Also, that these sigmoid models are empirical in the sense that the parameters have some biological interpretation. Comparisons were made among these models for goodness of fit. The 3 sigmoid models viz., Brody, Von-Bertalanffy and Gompertz over estimated the weights. Richards and Logistic models suited the data pattern. Logistic model in comparison with Richards model is computationally easier to fit. In the above said models parameters were estimated using ordinary least squares technique. But the assumptions for applications of this technique are early met in practical situation. So the parameters were estimated under heteroscedastic variance structure also the estimates so obtained were found to give better results.

Key words: Cattle, Growth pattern, Heteroscedastic variance structures, Sigmoid models

In animal science experiments, animals are generally taken as a subject and more than one observations are taken on the same animal, in general, at equal intervals of time as it makes the data symmetrical. These observations may be on body weight, body length, etc. These observations are called repeated measurements. The sequence of repeated measurements taken on an individual forms a time series, but it differs from time series data in the sense that the data are correlated. The observations that are closer in time are relatively more correlated than those widely spaced. Researchers in the field of behavioural and life sciences often come across with the studies on growth. Brody (1945) defined growth as relatively irreversible in magnitude of the measured dimension or function. Since a series of weight-age data points is analytically unwieldy and difficult to interpret. It is therefore desirable to study statistically the growth of animals. Statistical analysis of data on growth of animals involves choosing a suitable growth curve/model and proper method of pooling the results over the group of experiments. A growth curve is a mathematical relationship between the size of an animal and unit of time t. Then their relationship can be put as $y = f(t)$. Brown *et al.* (1976) compared 5 non-linear models viz., Von Bertalanffy, Logistic, Gompertz, Brody and Richards to fit weight-age data of beef and dairy cows and found that the Richards function has a variable point of inflection. They noticed minor differences between Richards and Logistic models. Doren *et al.* (1989) also considered Brody and Richards models for studying the growth of males. Beltran *et al.* (1992) compared growth pattern of 2 lines selected to predict mature weight and maturing rate by using Brody and Richards growth functions. Peretto *et al.* (1992) compared non-linear functions for describibng growth curves of three genotypes of female dairy cattle. These non-linear functions were compared through analysis of variance and residual mean square. The reliable function was then selected based on the estimates of analysed growth traits as well as the smallest root mean square error (RMSE). The Richards function was found to give a better fit to the actual growth data.

The method of ordinary least-squares (OLS) is generally used for parameter estimation in these studies but the conditions for OLS are very restrictive, particularly the assumption of constant variance for error term. When this assumption does not hold, the data is heteroscedastic rather than homoscedastic. Presently this situation prevails as the data showed heteroscedasticity when rank correlation test is applied and thus, a non-linear model with combined consideration for both heteroscedastic and inter-individual variance structures is tried in the present work.

MATERIALS AND METHODS

The data utilized in the present investigation were obtained

Present address: [1]Ph.D. Scholar, [2]Formerly Principal Scientist, [3]Scientist.

on crossbred cattle (25% Hariana, 25% Jersey, 50% Holstein-Friesians) maintained at Indian Veterinary Research Institute (IVRI), Izatnagar. The data were collected on cattle body weights from birth to 26 months of age at monthly interval. The data were collected on 25 animals.

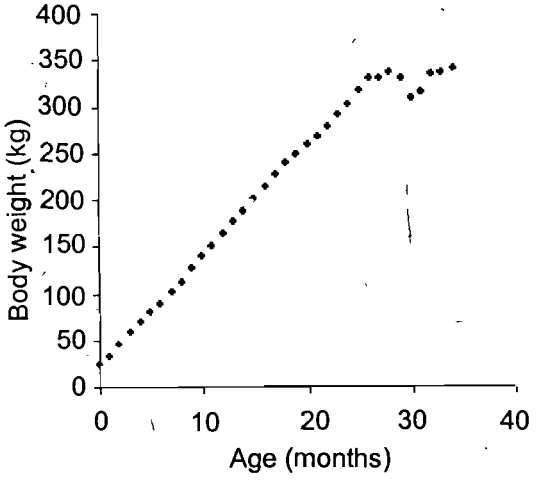
Growth model

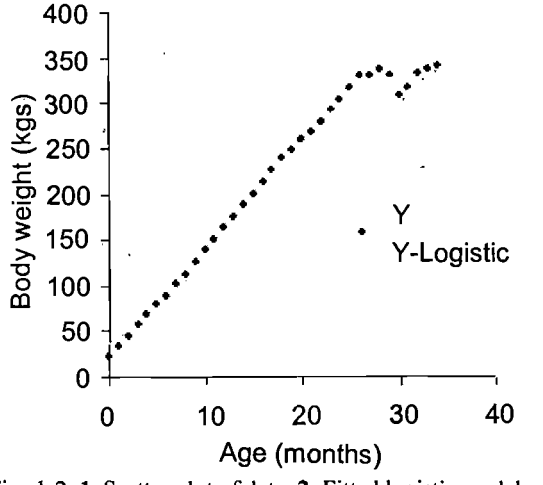
Consider the following model

$$y_j = f(x_j,\beta) + e_j \quad \ldots (1)$$

Usually, it is assumed that the errors e_j have zero means, common variance, are uncorrelated and normally distributed. However, as far as cattle growth is concerned, the assumption that errors are independently distributed with constant variance is rarely met in reality, particularly for growth models. When the assumption of constant intra-individual response variance is violated, the growth data exhibit constant coefficient of variation rather than constant variance (Davidian and Guiltinan 1995); i.e. variance is proportional to squares of the mean response. So a more appropriate assumption for model (1) would be;

$$E(y_j) = f(x_j,\beta),\ V(y_j)=(CV)^2 * [f(x_j, \beta)]^2$$





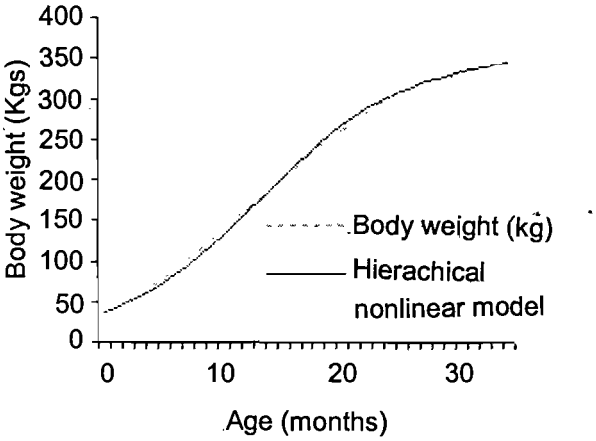
Figs 1-2. **1.** Scatter plot of date. **2.** Fitted logistic model.

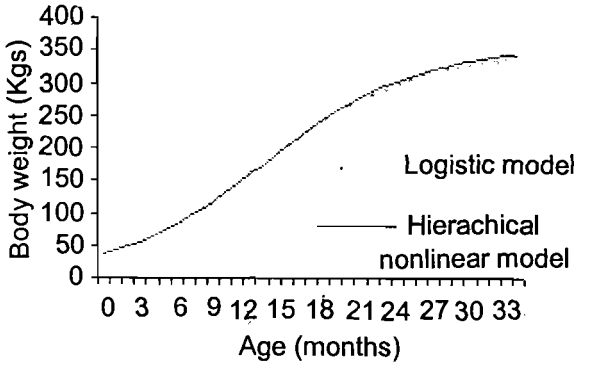
Since heterogeneity of variance is evident, hence ordinary least squares (OLS) estimation of ß may be inefficient relative to methods that consider heteroscedasticity using generalized least-squares (GLS) estimation. To motivate our discussion of this issue and of how the classical least-squares procedure may be modified, consider the situation where variance is non-constant across the response range such that the variances of y_j are known up to a constant of proportionality; that is:

$$E(y_j) = f(x_j,\beta),\ V(y_j)=(CV)^2/w_j \quad \ldots (2)$$

for some constants w_j, j = 1, 2, ..., n.

Under this setting with the assumption of independent, normal errors, it is straightforward to show that the maximum likelihood estimator ß is the value $\hat{\beta}_{WLS}$ that minimizes normal





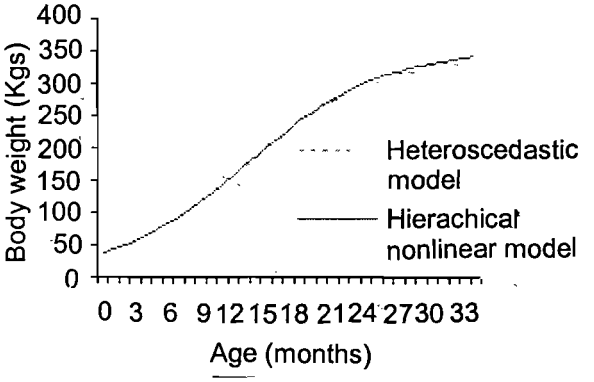


Fig. 3. a, b, c, Comparison of expected values by Hierarchical non-linear model with observed values, expected values of logistic model and heteroscedastic model.

equation,

$$\sum_{j=1}^{n} W_j\{Y_j - f(x_j,\hat{\beta})\}^2 \quad \ldots (3)$$

As ordinary least-squares, the maximum likelihood estimation for σ^2 is usually replaced by

$$\hat{\sigma}^2 WLS = (n-p)^{-1}\sum_{j=1}^{n} w_j\{y_j - f(x_j,\hat{\beta}\,WLS)\}^2 \quad \ldots (4)$$

where p is number of parameters to be estimated.

For definiteness, consider the constant coefficient of variation model (1). In this model, except for the multiplicative constant $(CV)^2$, variance is known up to the value of the regression parameter ß, which appears through the mean response. An obvious approach is thus to take advantage of the functional form for a variance to construct estimated weights, replacing ß by a suitable estimate, and to apply the weighted least-squares idea. The OLS estimator $\hat{\beta}_{OLS}$ is a natural choice to use for construction of estimated weights. Formally, an estimator for ß that takes into account the assumed mean-variance relationship may be obtained by forming estimated weights

$$\hat{w}_j = 1/[f(x_j,\hat{\beta}OLS)]^2 \quad \ldots (5)$$

and then solving (3), using the $\hat{w}_j$ in place of w_j. Intuition suggests that the resulting estimator for ß will be preferred to that obtained by ordinary least-squares.

Hierarchical non-linear models

The hierarchical non-linear models are staged models. At the first stage, inter-individual variations are characterized by a non-linear regression model with a model for the individual covariance structure. Inter-individual variability is represented in the second stage through individual-specific regression parameters, which may incorporate both systematic and random effects.

Let y_{ij} denote the j-th response $j = 1,2,...,n_i$ for i-th individual $i = 1, 2, ..., m$ taken at a set of conditions summarized by the vector of covariates x_{ij}, so that a total of

$$N = \sum_{i=i}^{m} n_i$$

responses have been observed. The vector x_{ij} incorporates variables such as a time. The function f(x,ß) may be specified to model the relationship between y and x, where ß is a (px1) vector of parameters. Although the form of f os common to all individuals, the parameter ß may vary accross individuals. This possibility is taken into account by specification of seperate (px1) vector of parameters $ß_i$ for the i-th individual. The mean response for individual i depends on the regression parameter $ß_i$ specific to that individual. This may be written as

Table 1. Non-linear summary statistics of different models

Model	Parameter	Estimate	Asymptotic std. error	Asymptotic 95% confidence interval		MAE	RMSE	R^2
				Lower	Upper			
Logistic	A	348.76	4.836	338.91	358.61			
	B	8.19	0.496	7.18	9.20			
	C	0.16	0.006	0.15	0.17			
						6.92	8.80	0.99
Richards	A	357.84	9.990	337.47	378.21			
	B	2.82	2.450	-2.17	7.81			
	C	0.13	0.020	0.09	0.18			
	D	0.57	0.300	-0.04	1.18			
						6.63	8.34	0.99
Gompertz	A	378.87	8.210	362.15	395.59			
	B	2.65	0.088	2.47	2.83			
	C	0.09	0.004	0.09	0.11			
						6.40	9.01	0.99
Von-Bertalanffy	A	401.77	11.997	377.33	426.20			
	B	0.62	0.016	0.59	0.65			
	C	0.08	0.004	0.07	0.08			
						12.82	15.34	0.99
Brody	A	552.89	53.724	443.36	662.32			
	B	0.99	0.011	0.96	1.01			
	C	0.03	0.004	0.02	0.04			
						11.76	13.20	0.98

Table 2. Rank correlation test for testing heteroscedasticity

X	yhat	Y	Error	|error|	Rank X	Rank |error|	Diff	(Diff)²
0	0	23	23	23	1	13	12	144
1	16	33	17	17	2	10	8	64
2	31	44	13	13	3	8	5	25
3	46	58	11	11	4	6	2	4
4	62	68	6	6	5	3	-2	4
5	77	80	3	3	6	1	-5	25
6	93	89	-3	3	7	2	-5	25
7	108	101	-7	7	8	4	-4	16
8	123	113	-11	11	9	5	-4	16
9	139	126	-12	12	10	7	-3	9
10	154	139	-15	15	11	9	-2	4
11	169	151	-19	19	12	11	-1	1
12	185	163	-22	22	13	12	-1	1
13	200	175	-25	25	14	14	0	0
14	216	188	-28	28	15	15	0	0
15	231	200	-31	31	16	16	0	0
16	246	213	-34	34	17	17	0	0
17	262	226	-36	36	18	18	0	0
18	277	239	-39	39	19	19	0	0
19	292	248	-45	45	20	20	0	0
20	308	258	-49	49	21	21	0	0
21	323	267	-56	56	22	22	0	0
22	339	278	-60	60	23	23	0	0
23	354	291	-63	63	24	24	0	0
24	369	302	-67	67	25	25	0	0
25	385	315	-69	69	26	26	0	0
26	400	328	-72	72	27	27	0	0
27	416	330	-86	86	28	28	0	0
28	431	336	-95	95	29	29	0	0
29	446	329	-118	118	30	30	0	0
30	462	308	-154	154	31	31	0	0
31	477	315	-162	162	32	33	1	1
32	492	333	-160	160	33	32	-1	1
33	508	335	-173	173	34	34	0	0
34	523	340	-183	183	35	35	0	0
			Rank =0.95			Total	0	340

Table 3. Comparison of expected values by hierarchical non-linear with the expected values by OLS and GLS models along with the observed values

Age (months)	Body weight (Kg)	Hierarchical non-linear model	OLS	GLS
0	23	37	38	38
1	33	43	44	44
2	44	49	50	50
3	58	56	57	57
4	68	64	65	65
5	80	73	74	74
6	89	83	84	84
7	101	94	95	95
8	113	106	106	106
9	126	118	118	118
10	*139*	*132*	*131*	*131*
11	151	145	145	144
12	163	159	158	158
13	175	174	172	172
14	188	188	186	186
15	200	202	200	200
16	213	216	213	213
17	226	230	226	226
18	239	242	239	239
19	248	254	251	250
20	258	266	262	261
21	267	276	272	271
22	278	285	281	280
23	291	294	289	289
24	302	302	297	296
25	315	308	303	303
26	328	315	309	309
27	330	320	315	314
28	336	325	319	319
29	329	329	323	323
30	308	332	327	327
31	315	335	330	330
32	333	338	333	332
33	335	340	335	335
Chi-square values		17.3	18.2	18.3

$$E(y_{ij}|\beta_i) = f(x_{ij}, \beta_i)$$

To handle the above, consider the following 2 stage model

Stage1 (intra-individual variation)

Assume that for individual i, the i-th response follows the model

$$y_{ij} = f(x_{ij}, \beta_i) + e_{ij}$$

Where e_{ij} is a random error term reflecting uncertainty in the response, given the i-th individual with $E(e_{ij}|\beta_i) = 0$. Collecting the responses and errors for the i-th individual into $(n_i x 1)$ vector $y_i = [y_{i1}, y_{i2}, \ldots, y_{ini}]'$ and $e_i = [e_{i1}, e_{i2}, \ldots, e_{ini}]'$, respectively, and defining the $(n_i x 1)$vector

$$f_i(\beta_i) = \begin{bmatrix} f(x_{il}, \beta_i) \\ . \\ . \\ . \\ f(x_{in_i}, \beta_i) \end{bmatrix}$$

we may summarize the data for the i-th individual as

$$y_i = fi(\beta_i) + e_i$$

where

$$E(e_{ij}|\beta_i) = 0$$

Table 4. OLS and GLS parameter estimates

Animal	A		B		C	
	OLS estimate	GLS estimate	OLS estimate	GLS estimate	OLS estimate	GLS estimate
1	331.70	317.90	14.01	0.15	8.39	8.78
2	370.70	343.70	20.75	0.15	7.77	8.26
3	317.20	290.00	9.25	0.19	8.17	8.43
4	310.50	290.10	8.90	0.16	8.58	8.98
5	367.50	290.10	23.35	0.16	8.58	9.66
6	380.60	351.60	8.50	0.17	9.53	9.87
7	400.10	375.40	14.69	0.16	8.57	8.99
8	726.20	647.00	63.37	0.13	13.68	15.01
9	407.50	409.60	20.07	0.14	8.88	9.10
10	549.10	504.60	78.06	0.11	10.36	11.29
11	496.40	446.60	28.50	0.14	9.26	10.01
12	337.00	298.90	9.90	0.16	8.31	8.72
13	367.50	357.90	18.27	0.13	8.10	8.43
14	411.70	345.60	13.38	0.16	8.91	9.53
15	381.00	330.10	14.72	0.16	6.66	7.16
16	367.10	323.60	10.73	0.19	6.44	6.70
17	314.10	290.20	10.94	0.17	5.81	6.20
18	384.10	353.30	19.59	0.17	6.51	6.91
19	393.20	378.60	22.90	0.14	9.16	9.56
20	351.20	256.40	16.05	0.18	5.04	5.53
21	364.60	341.70	11.27	0.18	7.69	8.04
22	336.40	310.90	10.87	0.19	6.74	7.04
23	367.60	283.30	17.42	0.18	6.80	7.18
24	321.70	234.20	11.25	0.25	8.98	6.72
25	370.50	340.30	16.37	0.15	8.78	9.32

The model describes the systematic and random variation associated with measurements on the i-th individual. Systematic variation is characterized through the regression function f, while random variation is represented by an assumption on the random errors e_i. Specification of a model for the distribution of the e_i completes the description of intra-individual variation for the i-th individual.

Stage 2 (inter-individual variation)

Variation among different individuals is accounted for through the individual-specific regression parameters $ß_i$. Part of the inter-individual variation in parameter values may be due to systematic dependence on individual characteristics, such as weight, or age. To account for inter-individual variation among the $ß_i$, the standard approach is to specify a model for the $ß_i$. The degree of complexity of this model will depend on the nature of the data. Variation among the parameter values of the function across subjects is generally attributable mainly to random variation among subjects rather than to differences in individual demographic and physiological characteristics. In both these situations a model, that assumes inter-individual variation due entirely to unexplained phenomena, can be expressed as:

$$ß_i = ß + b_i \quad \ldots (7)$$

Where ß is a (px1) vector of fixed parameters and b_i is (px1) random effect assumed to arise from a population with mean 0 and covariance matrix D. The above equation implies that the individual specific regression parameters have mean ß. The covariance matrix D quantifies the random inter-individual variation.

Standard two-stage (STS) method

In the simplest case where, the b_i's are independent and identically distributed, STS estimate are constructed as the sample mean and covariance of the $ß_i$:

$$\hat{\beta}_{STS} = m^{-1}\sum_{i=i}^{m} \beta_i$$

$$\hat{D}_{STS} = (m-1)^{-1}\sum_{i=i}^{m} (\beta_i - \hat{\beta}_{STS})(\beta_i - \hat{\beta}STS)'$$

RESULTS AND DISCUSSION

The idea behind the present work is to give best-fitted model for the cattle weight to study the growth pattern of cattle. In order to have an idea about how the cattle weight behaves over time, the data were plotted. The scatter plot in Fig. 1 showed an 'S' shaped curve. So 5 different sigmoidal models were fitted to growth data. The values of the parameter

estimates with their asymptotic standard error and 95% confidence intervals along with R^2, RMSE and MAE for comparison are presented in Table 1. It is concluded that the model, which has minimum RMSE and minimum MAE, will be the best-fitted model. Based upon the criteria, the Richards and Logistic models fit equally well. Since Richards model is a four parameter model and it has variable point of inflection as compared to those of the Logistic model. Furthermore the standard errors of the parameters of Logistic model are lower in comparison to that of Richards model. Also the difference between the RMSE and MAE values is very small as compared to other models. So it was decided that the Logistic model best represented the system.

The brody's model underestimates the weight at early stages of the animal. Here, we predict the values using ordinary least-squares (OLS) estimation. In OLS estimation procedure, it is assumed that the error variance is constant or a homoscedastic error structure. In case of animal growth data, many a time, the above assumption is violated. In the present study too, data revealed heteroscedastic, the OLS is not to be used any more. So, to estimate the parameters we go for using the procedure explained earlier. For the procedure discussed, a computer program has been developed in IML (Interactive Matrix Language) module of SAS and the parameters under heteroscedastic error structure are estimated. The same program can be used for estimating the parameters through inter-individual variance. The predicted values are calculated using both estimates of parameters i.e., using homoscedastic and heteroscedastic structures. From the analysis it was revealed that the values of RMSE of logistic models in homoscedastic and heteroscedastic error structures are 8.80 and 8.51 respectively. Hence, from these values, it was concluded that the logistic model in heteroscedastic structure is more stable than that of logistic model in homoscedastic structure. Fig. 2 shows the graphical comparison of expected values by Logistic model to observed values.

Since the errors can be classified into systematic errors or random errors as discussed earlier, we calculate the inter-individual variance structure. For this, we first calculate the parametric values of each individual through OLS procedure. The parameter estimates are given in Table 2. Moreover, after calculating these OLS estimates we again estimate these parameters through GLS and the parameter values are also presented in Table 4. Now, we average over the 25 parameter vector to get the estimated parameter vector. It can be observed from Table 3 that the estimates are nearer to the original values than those of OLS and GLS. Fig. 3 depicts the graphical comparison of the predicted values using OLS, GLS variance structure and hierarchical non-linear model. So for better prediction one can be advised to use the hierarchical non-linear model instead of OLS or GLS models.

To summarize, it is found through graphical and empirical comparisons that the Logistic model best suits the animal growth data and as the data were heteroscedastic in nature, the GLS was applied and found that these estimates out perform the OLS estimates. To classify the errors, hierarchical non-linear model is used and found that parameter estimates are the best among the three viz. OLS, GLS and hierarchical non-linear model.

REFERENCES

Beltran J J, Butts W T, Olson T A and Koger K. 1992. Growth patterns of two lines of Angus cattle selected using predicted growth parameters. *Journal of Animal Science* **70**: 734.

Brody S. 1945. *Bioenergetics and Growth.* Rheinhold Publishing.

Brown J E, Fitzhugh H A Jr and Cartwritht T C. 1976. A comparison of non-linear models for describing weight-age relationship in cattle. *Journal of Animal Science* **42**: 810.

Davidian D and Giltinan M. 1995. *Non-linear Models for Repeated Measurement Data.* London Chapman Hall.

Doren P E, Bakef J F, Long C R and Cartwright. 1989. Estimating parameters of growth curves of bulls. *Journal of Animal Science* **67**: 1432.

Peretto D, Cue R I and Lee A J. 1992. Comparison of non-linear functions for describing growth curves of three genotypes of dairy cattle. *Canadian Journal of Animal Science* **72**: 773.

Indian Journal of Animal Sciences 73 (10): 1180-1181, October 2003

Genetic studies on breeding efficiency in crossbred cows

DEEPAK KUMAR[1] and D KUMAR[2]

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttaranchal 263145 India

Received: 20 April 2002; Accepted : 10 June 2003

Key words: Animal production, Breeding efficiency, Cattle

The investigation was undertaken to know the inheritance pattern for the traits - breeding efficiency (BE), weight at first calving (WFC), first service period (FSP), first gestation period (FGP), first lactation period (FLP), first calving interval (FCI) and first lactation milk yield (FLMY) in ½ Jersey × ½ Sahiwal cows.

Data on 116 half Jersey half Sahiwal cows, maintained at Livestock Research Centre of the University, spread over a period of 8 years from 1972 to 1979, under 9 sires were utilized after adjusting for year, season and age group effects, using least-squares technique according to Harvey (1987). The heritability was estimated by paternal half-sib method as described by Hazel and Terril (1945). The genetic correlations were estimated by the method given by Hazel *et al.* (1943) and Phenotypic correlations as described by Searle (1961).

The standard error of heritability, genetic correlation and phenotypic correlation were computed as given by Swiger *et al.* (1964), Hazel *et al.* (1943) and Panse and Sukhatme (1967) respectively.

The breeding efficiency was computed as $BE=[(n.365)+900/A_c+C_i] \times 100$, where, n = number of calving intervals, A_c = the actual age at first calving in days and C_i = the sum of the actual calving intervals in days.

The least-squares estimates (means ±SE) for various traits along with heritability values are given in Table 1. Similar estimates were reported by Daspurakayastha and Mazumdar (1979) for breeding efficiency in Hariana × Jersey crossbreds; Singh *et al.* (1990) in Sahiwal × Jersey crossbreds for weight at first calving; Prasad *et al.* (1991) in Sahiwal × Jersey crossbreds for first service period; Suresh Chand and Sharma (1985) for first calving interval in Sahiwal breed; Roy *et al.* (1987) for first lactation period in Sahiwal × Jersey crossbreds and Suresh Chand and Sharma (1985) in Sahiwal × Jersey cows for first lactation milk yield. Abraham (1973) had reported lower estimate of BE. Lower BE estimates might be due to higher age at first calving, longer service period or longer calving intervals. Present results and available reports indicate that in Sahiwal × Jersey crossbreds service period can be controlled easily near-about 100 days and efforts should be diverted towards reducing it.

Table 1. Least-squares means with standard errors for different economic trait

Traits	Mean ± S E	Heritability±SE
Breeding efficiency (%)	82.06±1.12	0.285±0.21
Weight at first calving (kg)	328.97±5.77	0.314±0.022
First service period (days)	151.07±13.23	0.229±0.018
First gestation period (days)	276.79±1.69	0.208±0.017
First lactation period (days)	312.32±14.34	0.167±0.015
First calving interval (days)	440.34±15.92	0.110±0.012
First lactation milk yield (kg)	2662.67±132.90	0.116±0.012

The BE exhibited relatively higher heritability in comparison to other reproductive traits. The production traits indicated lower genetic base. Thus some emphasis may be given to this trait while selecting dairy cows for milk production and better reproductive performance. Similar heritability value was reported by Sandhu (1986) in Sahiwal × Jersey crossbreds for BE and Suresh Chand and Sharma (1985) for FCI in Sahiwal breed.

The genetic correlations (r_G) of BE with WFC and BE with FLP were more than 1. Cows having higher BE had shorter service period. The r_P of BE with FCI indicated the increase in BE for shorter calving interval. The negative and significant r_G and r_P value between BE and FLP could be due to the fact that longer FLP might increase FCI and hence reduced the BE. The r_G of BE with FGP was negative and significant, whereas r_P of BE with FGP was insignificant. The phenotypic variation in the trait FGP was not much more.

Singh (1978) also observed positive and significant r_G of BE with WFC but insignificant value of r_P for this combination in Sahiwal breed. The reports given by Singh *et al.* (1980) for r_G and r_P of BE with FSP in Sahiwal breed; Gandhi and Gurnani (1988) in reference to r_G of BE with FCI in Sahiwal, and Singh (1978) in reference to r_G and r_P of BE with FLP were in agreement with these results. The r_G and r_P values of BE with FLMY were significant and negative in direction.

Present address: [1]Research Scholar, [2]Associate Professor, Department of Genetics and Animal Breeding, College of Veterinary Sciences.

Table 2. Genetic and phenotypic correlations of different traits with breeding efficiency

Correlations	Traits					
	WFC	FSP	FGP	FLP	FCI	FLMY
r_G	-1.309**±	-0.779**±	-0.986**±	-1.173**±	0.514**±	-0.818**±
	0.084	0.067	0.077	0.088	0.022	0.081
r_P	-0.313**±	-0.205*±	-0.082±	-0.207**±	-1.319**±	-0.201*±
	0.084	0.089	0.092	0.089	0.083	0.089

*Significant at 5% level of significance; **significant at 1% level of significance.

Singh *et al.* (1980) also reported the positive and significant r_G of BE with FLMY in Sahiwal cows.

The significant r_G and r_P of FLMY with BE may be due to the fact that service period affect lactation production adversely resulting in lesser milk yield in first lactation which, however, may be compensated through an increase in life time production in terms of increase in number of lactations. The cows calving at an early age too may not have sufficient body weight to sustain higher milk yield in first lactation.

SUMMARY

This study revealed that the cows having higher breeding efficiency have lower weight at first calving, shorter service period and calving interval. The reduction in service period, which is desirable, should be brought about through planned management.

REFERENCES

Abraham. K C. 1973. 'Effects of cross breeding on reproduction and production performance of dairy cattle.' M. Sc. (Ag.). Thesis. HAU, Hisar.

Brijendra Singh. 1978. 'Studies on breeding efficiency in Sahiwal, Sahiwal Friesian half breds.' M.V. Sc. Thesis. IVRI, Izatnagar, Bareilly.

Brijendra Singh, Bhat P N and Kumar S. 1980. Factors affecting breeding efficiency in Sahiwal cows. *Indian Journal of Animal Sciences* **50**: 915-29.

Daspurakaystha P C and Mazumder S C. 1979. A note on milk production and reproductive efficiency in a dairy herd. *Indian Journal of Animal Sciences* **49**: 66-67.

Gandhi R S and Gurnani M. 1988. Association amongst different productive and reproductive traits in Sahiwal cattle. *Asian Journal of Dairy Research* 7: 171-74.

Harvey W R. 1987. *Least-squares Analysis of Data with Unequal Subclass Numbers*. ARS, US Department of Agriculture.

Hazel L N, Baker M I and Reinmitter C F. 1943. Genetic and environmental correlation between growth rate of pigs at different ages. *Journal of Animal Science* **2**: 118-28.

Hazel L N and Terril C E. 1945. Heritability of weaning weight and staple length in range Rambouillet lambs. *Journal of Animal Science* **4**: 347-58.

Panse V G and Sukhatme P V. 1967. *Statistical Methods for Agricultural Workers*. ICAR Publication, New Delhi.

Prasad V S, Rao G N, Satyanarayana A, Rao M R and Jayaram Krishna V. 1991. Performance of indigenous and crossbred cow under village condition in Chittor district of Andhra Pradesh. *Indian Journal of Dairy Science* **44**: 196-99.

Roy R, Tomar S P S, Sharma R J, Manglik V P and Chaudhary R P. 1987. Additive and heterotic effects on economic traits in Sahiwal × Jersey crossbred cattle. *Indian Journal of Animal Science* **57**: 297-300.

Sandhu A S. 1986. Genetic studies on age at first calving and breeding efficiency and their association with life time milk production in crossbreds. M.Sc. (Ag.) Thesis. GBPUA & T, Pantnagar.

Searle S R. 1961. Phenotypic, genetic and environmental correlations. *Biometrics* **17**: 474-80.

Singh V P, Singh R V, Singh C V and Singh S P. 1990. Genetic studies on reproductive efficiency traits in Sahiwal and its crosses with Jersey and Red Dane. *Indian Journal of Animal Sciences* **60**: 90-92.

Suresh Chand and Sharma O P. 1985. Inheritance of persistency of milk yield and its relationship with production traits among Sahiwal and × ½ Sahiwal 1/2 Jersey cows. *AASP Animal Science Congress Seol (Korea)* 6-10 May.

Swiger L A, Harvey W R, Everson D O and Gregory K E. 1964. The variance of interclass correlations including groups with one observation. *Biometrics* **20**: 818-24.

Indian Journal of Animal Sciences 73 (10): 1182-1183, October 2003

Preweaning growth in indigenous pigs of eastern region*

K SUDHAKAR[1] and G K GAUR[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received : 15 November 2002; Accepted : 2 June 2003

Key words: Genetic parameters, Indigenous pigs, Pre-weaning growth

Rapid growth in pigs is considered a major component of profit determination and also pre-weaning growth has been noticed to have close association with body weights at later stages (Thomson *et al.* 1947) and thus, helpful for selection or judicious culling at an early stage of life. The indigenous pigs due to natural selection over the years are famous for adaptation to local environment, heat tolerance, disease resistance and maintenance on low inputs. The present investigation was therefore undertaken on 481 indigenous piglets that were progeny of 41 boars and 111 gilts and born to 6 generations from 1983 onwards, to determine the pre-weaning growth performance of indigenous pigs reared at All-India Co-ordinated Research Project on Pigs, Khanapara, Assam, India. Piglets were reared along with dams and were weaned at 56 days of age. Data were classified according to generation, sire, dam and sex of the piglet and analyzed using Mixed Model Least Squares and Maximum Likelihood Computer Programme (Harvey 1987) using following model:

$$Y_{ijklm} = \mu \pm G_i \pm S_{ij} \pm D_{ijk} \pm SX_l \pm b_{ijklm} \pm eijklm$$

Where, Y_{ijklm} = observations on m^{th} progeny of j^{th} sire belonging to i^{th} generation; μ = overall mean, Gi = fixed effect of i^{th} generation, S_{ij} = random effect of j^{th} sire within i^{th} generation, D_{ijk} = random effect of k^{th} dam within j^{th} sire and i^{th} generation, SX_l = fixed effect of l^{th} sex, b_{ijklm} = partial regression of observation on age of dam at farrowing and e_{ijklm} = random error.

The least-squares mean of the weekly body weights and average daily weight gain (ADWG) has been presented in Table 1. The average body weight ranged from 0.853 ±0.014 kg at birth to 7.421±0.135 kg at 8th week of age. The daily weight gain averaged 116.788±2.400 g. The average weights at different ages in the present study were comparable with the results of Krishan Lal *et al.* (1988).

The analysis of variance for weekly body weights and ADWG revealed significant effect ($P \leq 0.01$) of generation on all the traits. The birth weight improved initially from generations 1 to 2 and declined thereafter. The significant difference between generations could be due to yearly changes in environmental conditions, managemental practices and also inbreeding that could have played a role in the herd being very closed one. A similar significant effect of year of birth of piglets on body weights during preweaning growth was observed by Shylla *et al.* (1991) in indigenous pigs.

Nonsignificant effect of sire and significant effect of dam on all body weights and ADWG were observed. This revealed the existence of large maternal influence arising from natural variation in the prenatal and pre-weaning nutrition and environment on pre-weaning growth and small additive genetic variance.

In the present study average age of dam at first farrowing was 437.83±3.05 days. The regression coefficients of the weekly body weights and ADWG on dam's age at first farrowing were nonsignificant ($P \leq 0.01$) but positive. Effect of sex was nonsignificant ($P \leq 0.05$) on all body weights except at birth and one-week age. Average daily weight gain was also free from this effect. Nonsignificant effect of sex during pre-weaning period could be due to non-release of sex hormones during this stage.

Heritability estimates for most of the weights were low ranging from 0.01 to 0.23. The heritability estimates were 0.010 ± 0.092, 0.455 ± 0.171, 0.337 ± 0.152, 0.229 ± 0.139, 0.120 ± 0.114, 0.079 ± 0.106, 0.120 ± 0.114 for weekly body weights from second to eighth weeks respectively. These estimates revealed the little existence of additive genetic variance in growth traits that could further be exploited using progeny testing scheme in the herd. Existence of substantial amount of variance due to common environment provided by dam was also confirmed from these estimates. The estimates of heritability for body weights at birth and first week and some genetic correlations could not be calculated due to negative sire components that were set to zero in the program. This could be due to sampling error, selected group

*Part of M.V.Sc. Thesis submitted by first author to Indian Veterinary Research Institute, Izatnagar, Bareilly.

Present address: [1]Veterinary Assistant Surgeon, Regional Animal Husbandry Training Center (CSCC, Campus) Gannavaram 521 101, Andhra Pradesh.

[2] Senior Scientist, National Bureau of Animal Genetic Resource, Karnal, Haryana 132 001.

Table1. Least-squares means for body weights (kg) and average daily weight gain (g) across the generation and sex

Effect	Observations	Body weight at									
		Birth	1 week	2 week	3 week	4 week	5 week	6 week	7 week	8 week	ADWG
m	481	0.853 ± 0.014	1.673 ±0.030	2.559 ±0.047	3.413 ±0.066	4.166 ±0.079	4.888 ±0.091	5.640 ±0.097	6.440 ±0.108	7.421 ±0.135	116.788 ±2.400
Generation	144	0.755 ±	1.424 ±	2.383 ±	3.228 ±	4.090 ±	4.811 ±	5.471 ±	5.970 ±	6.746 ±	106.454 ±
1		0.033[b]	0.069[b]	0.107[b]	0.143[b]	0.174[bc]	0.201[b]	0.218[b]	0.243[b]	0.304[b]	5.373[b]
2	147	0.918 ±	1.850 ±	2.834 ±	3.664 ±	4.439 ±	5.267 ±	6.114 ±	7.029 ±	7.029 ±	129.429 ±
		0.026[a]	0.054[a]	0.084[a]	0.129[a]	0.153[a]	0.173[a]	0.180[a]	0.197[a]	0.250[a]	4.500[a]
3	36	0.915 ±	1.823 ±	2.692 ±	3.580 ±	4.256 ±	5.065 ±	5.847 ±	6.791 ±	8.018 ±	126.306 ±
		0.050[a]	0.103[a]	0.159[a]	0.215[a]	0.261[ab]	0.302[ab]	0.326[a]	0.362[a]	0.454[a]	8.032[a]
4	41	0.869 ±	1.614 ±	2.396 ±	3.104 ±	3.779 ±	4.326 ±	5.008 ±	5.860 ±	6.727 ±	104.162 ±
		0.043[a]	0.088[bc]	0.137[b]	0.181[b]	0.221[c]	0.256[c]	0.279[b]	0.310[b]	0.389[b]	6.853[b]
5	52	0.875 ±	1.684 ±	2.714 ±	3.610 ±	4.556 ±	5.437 ±	6.361 ±	7.266 ±	8.285 ±	131.756 ±
		0.039[a]	0.080[ac]	0.124[a]	0.160[a]	0.196[a]	0.228[a]	0.251[a]	0.280[a]	0.350[a]	6.149[a]
6	61	0.783 ±	1.645 ±	2.333 ±	3.289 ±	3.876 ±	4.420 ±	5.034 ±	5.723 ±	6.555 ±	102.626 ±
		0.041[b]	0.085[b]	0.131[b]	0.178[b]	0.216[c]	0.250[c]	0.269[b]	0.299[b]	0.376[b]	6.648[b]
Sex	243	0.864 ±	1.699 ±	2.572 ±	3.424 ±	4.205 ±	4.937 ±	5.667 ±	6.458 ±	7.415 ±	116.458 ±
Male		0.015	0.032	0.050	0.070	0.085	0.098	0.106	0.118	0.460	2.593
Female	238	0.840 ±	1.647 ±	2.545 ±	3.400 ±	4.127 ±	4.839 ±	5.611 ±	6.421 ±	7.428 ±	117.119 ±
		0.015	0.032	0.050	0.070	0.085	0.098	0.106	0.117	0.146	2.581
Regression (L.S.constants)		0.000 ±	0.005 ±	0.002 ±	0.003 ±	0.006 ±	0.012 ±	0.013 ±	0.012 ±	0.003 ±	0.046 ±
Dams age at first farrowing		0.002	0.004	0.008	0.010	0.013	0.015	0.018	0.020	0.023	0.421

Means with same superscript do not differ significantly.

of sires, relationship among sires, confounding of sires within year and genotype × environment interactions. The heritability estimate of preweaning ADWG gain was 0.171 ± 0.123. All the phenotypic correlations are significant ($P< 0.01$). Phenotypic correlations among body weights ranged from 0.25 to 0.95. ADWG had highest phenotypic correlation (0.99) with weaning weight. This revealed that weaning weight could be effectively predicted from daily weight gain even at early age. Genetic correlations were high between weights at adjacent ages than non-adjacent ages and tended to decrease with increase in age.

SUMMARY

Preweaning body weights on 481 indigenous piglets revealed that the average body weight ranged from 0.853±0.014kg at birth to 7.421±0.135 kg at weaning and average daily weight gain as 116.788±2.400 g. Generation and dam has significant effect on all the traits. Whereas non-significant effect of sire and dam's age at first farrowing on all body weights and ADWG were observed. Effect of sex was nonsignificant on all body weights and ADWG except on weight at birth and one-week age. Heritability estimates for most of the weights were low ranging from 0.01 to 0.23.

ACKNOWLEDGEMENTS

I sincerely acknowledge the guidance of Dr R L Arora, Senior Scientist, AICRP (Pigs), IVRI, Izatnagar, in completing this research work, and the Director, Indian Veterinary Research Institute, Izatnagar, for providing financial assistance in the form of junior fellowship.

REFERENCES

Harvey R Walter. 1987. *Users Guide for LSMLMW PC-1 Version of Mixed Model Lest-squares and Maximum Likelihood Computer Program. Ohio,* 43320, USA.

Krishan Lal, Sharma G C, Mishra R R and Shiv Prasad 1988. Growth rate of indigenous pigs. *Indian Journal of Animal Sciences* **58**: 1116-20.

Shylla B, Bardoli T, Das D and Goswami R N. 1991. Growth rate of indigenous pigs of Assam as affected by some non-genetic factors. *Indian Veterinary Journal* 68: 232-34.

Thomson W, Biggar W A and Cook R. 1947.Nutrition and other factors in rearing of piglets. *British Journal of Nutrition.* 1: 11 (*fide Nutrition Abstracts and Reviews* 18: 1465.)

Indian Journal of Animal Sciences 73 (10): 1184-1186, October 2003

Improving the digestibility of finger millet straw by strategic supplementation through locally available concentrate ingredients and green fodders/top feeds

M CHANDRASEKHARAIAH[1], K T SAMPATH[2], U S PRAVEEN[3] and C PRAKASH[4]

National Institute of Animal Nutrtion and Physiology, Adugodi, Bangalore, Karnataka 560 030 India

Received : 5 November 2002; Accepted : 24 June 2003

Key words: Animal nutrition, Concentrates, Digestibility, Fodder

One of the methods to improve the digestibility of the crop residues is through supplementation of limiting nitrogen to provide suitable rumen environment for fibre digestion. The present study was therefore undertaken to determine the optimum level and source of locally available concentrate ingredients and green fodders/top feeds for improving the *in vitro* digestibility of finger millet straw (FMS).

Supplementation

The FMS was supplemented with groundnut-cake, sunflower-cake, deoiled rice bran (DORB), maize gluten meal-60 (60% CP) (MGM-60), maize gluten meal-40 (40% CP) (MGM-40), safflower extraction, cotton seed extraction, wheat bran, copra-cake, lucerne, maize fodder, sorghum fodder, para grass and subabul at 15, 22 and 30 g rumen degradable nitrogen (RDN) per kg digestible organic matter (DOM). In case of supplements with low RDN content urea was added to make up the deficit in providing required amount of RDN and to maintain the level of supplement to less than or equal to 50% of the total diet. The RDN content of supplements were calculated from the previously estimated ruminal degradability values for these feeds (Sampath *et al.* 1999). The RDN provided by straw was not taken into account for calculating the total RDN of the diet.

In vitro *organic matter digestibility (IVOMD)*

Oven dried samples of FMS and other supplements ground in a laboratory mill through a sieve with mesh of 2mm size were analyzed for organic matter and nitrogen (AOAC 1990). Rumen liquor was collected from 3 adult crossbred steers fed concentrate mixture (maize 30, groundnut-cake 25, wheat bran

Table 1. *In vitro* organic matter digestibility of finger millet straw (FMS) with concentrate ingredients and green fodder/g feed at different RDN levels

FMS without supplements	Supplements	With supplement			
		Level of RDN (g/kg DOM)			
		15	22	30	Mean
52.76^{a}±0.19	Groundnut-cake	59.91^{b}± 0.11	60.11^{b}± 0.27	62.27^{c}± 0.09	60.76^{w}± 0.76
52.76^{a}± 0.23	Sunflower-cake	54.75^{b}± 0.15	54.73^{b}± 0.14	55.91^{b}± 0.08	55.13^{u}± 0.39
52.76^{a}± 0.08	Deoiled rice bran and urea	56.80^{b}± 0.11	56.04^{b}± 0.15	57.25^{b}± 0.13	56.70^{v}± 0.35
52.76^{a}± 0.01	Maize gluten meal–60	64.86^{b}± 0.08	70.42^{c}± 0.01	71.78^{c}± 0.11	69.02^{z}± 2.12
52.76^{a}± 0.13	Maize gluten meal -40	61.84^{b}± 0.19	65.66^{c}± 0.35	69.83^{d}± 0.02	65.84^{y}± 2.31
52.76^{b}± 0.12	Safflower extractions	50.91^{a}± 0.10	51.11^{a}± 0.16	50.03^{a}± 0.25	50.68^{s}± 0.33
52.76^{a}± 0.12	Cotton seed extractions	60.14^{b}± 0.24	61.37^{b}± 0.01	61.22^{b}± 0.16	60.91^{w}± 0.39
52.76^{a}± 0.13	Wheat bran	51.15^{a}± 0.05	52.78^{a}± 0.09	54.85^{b}± 0.02	52.93^{u}± 1.07
52.76^{a}± 0.33	Copra-cake and urea	60.91^{b}± 0.09	61.18^{b}± 0.06	68.80^{c}± 0.09	63.63^{x}± 2.59
52.76^{a}± 0.45	Lucerne	65.08^{b}± 0.29	68.76^{c}± 0.14	69.91^{c}± 0.06	67.92^{z}± 1.46
52.76^{a}± 0.58	Maize fodder	57.61^{b}± 0.13	61.24^{c}± 0.13	62.44^{d}± 0.26	60.43^{w}± 1.45
52.76^{a}± 0.26	Sorghum fodder and urea	54.50^{b}± 1.9	54.91^{b}± 0.12	54.98^{b}± 0.30	54.80^{w}± 0.15
52.76^{a}± 0.10	Para grass and urea	53.96^{b}± 0.10	53.26^{ab}± 0.24	53.98^{b}± 0.14	53.73^{v}± 0.24
52.76^{a}± 0.09	Subabul and urea	63.86^{b}± 0.19	65.85^{c}± 0.45	65.19^{c}± 0.41	64.97^{y}± 0.59

a, b, c, d values within the row differ significantly (P<0.01); -s, t, u, v, w, x, y, z values within the column differ significantly (P<0.01).

Present address: [1]Scientist, [2]Principal Scientist, [3,4]Senior Research Fellow.

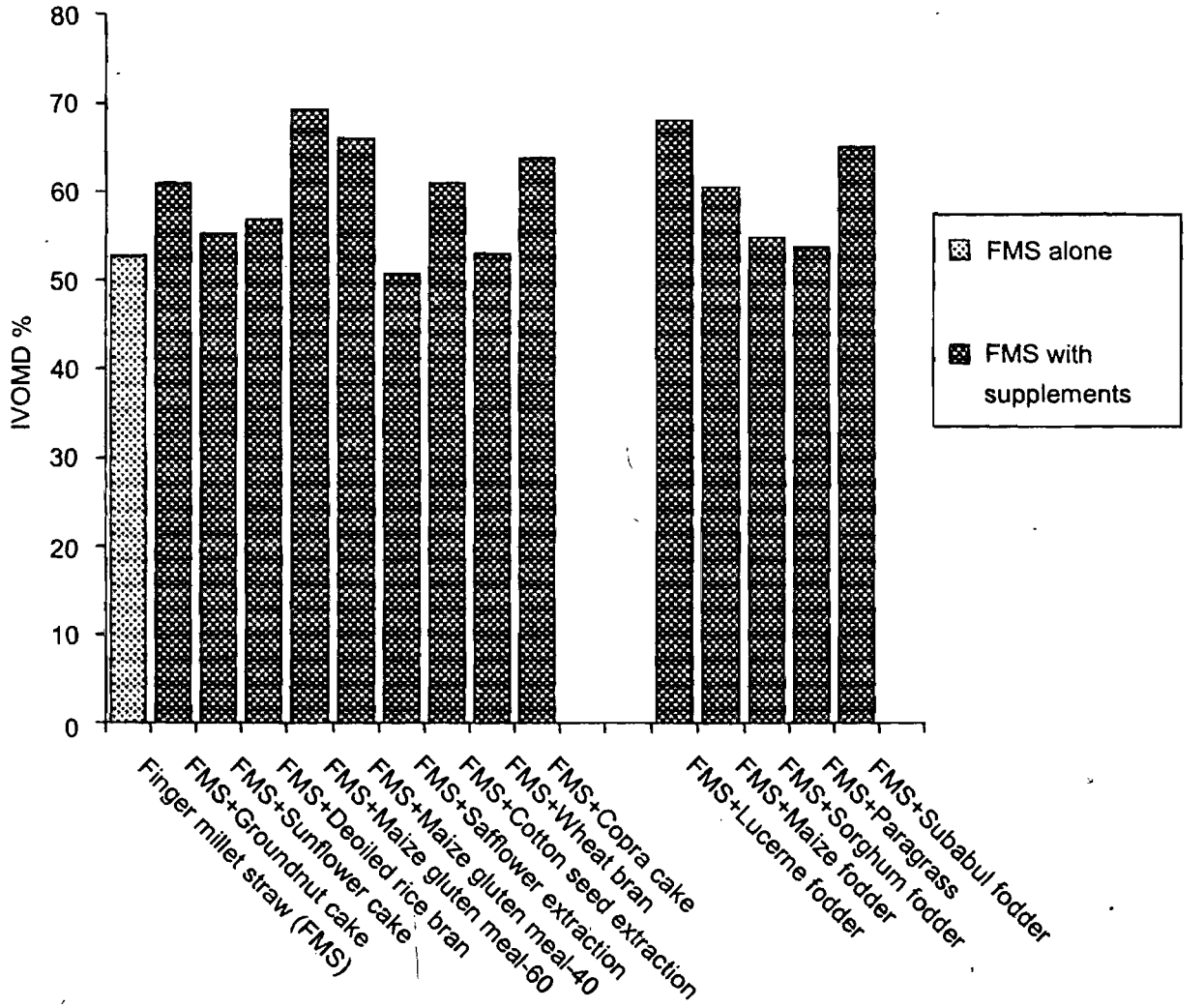


Fig. 1. *In vitro* organic matter digestibility of finger millet straw (FMS) supplemented with different concentrate ingredients and green fodders/top feeds.

42, mineral mixture 2 and common salt 1%) and *ragi* straw to meet their nutrient requirement for maintenance. The IVOMD of FMS with and without supplements (quadruplicate for each level and for each feed) was determined by the method of Van Soest *et al.* (1966) and was completed in 2 stages (a) fermentation with rumen liquor and (b) extraction with neutral detergent solution. The data were analysed statistically (Snedecor and Cochran 1968).

The IVOMD of finger millet straw was 52.76% and it was increased by 13.55, 13.93 and 18.95% due to the supplementation with groundnut-cake, 3.77, 3.73 and 5.97% with sunflower-cake, 7.67, 6.22 and 8.51% with DORB, 22.93, 33.47 and 36.05% with MGM-60, 17.21, 24.45 and 32.35% with MGM-40, 13.99, 16.32 and 16.03% with cottonseed extraction, 15.45, 15.96 and 30.40% with copra-cake, 23.35, 30.33 and 32.49% with lucerne, 9.19, 16.07 and 18.35% with maize fodder, 3.30, 4.08 and 4.21% with sorghum fodder, 2.27, 0.95 and 2.31% with para grass and 21.04, 24.81 and 23.56% with subabul at 15, 22 and 30g RDN/kg DOM supplementation respectively (Table 1). The increase in OM digestibility of finger millet straw by supplementation with the above feed ingredients could be attributed to the rate of rumen NH_3-N production providing desired rumen environment for microbes to stimulate straw digestion. No significant difference in OMD due to different levels of RDN was observed for concentrate ingredients such as sunflower extraction, deoiled rice bran, cottonseed extraction, sorghum fodder and para grass suggesting that providing 15g RDN/kg DOM may be adequate for FMS with these supplements as against 30 g RDN/kg DOM advocated by ARC (1980). The IVOMD of FMS was higher ($P<0.01$) at 22 and 30g RDN/kg DOM in case of MGM-60, lucerne and subabul suggesting that a minimum of 22g RDN is required to improve OM digestibility of FMS with these supplements. However, supplementation with groundnut-cake, copra-cake, MGM-40, wheat bran, and maize fodder at 30g RDN levels significantly ($P<0.01$) increased the IVOMD.

The mean OM digestibility of FMS was significantly

($P<0.01$) higher on MGM-60 supplementation followed by MGM-40, copra-cake, groundnut-cake, cottonseed extraction, DORB, and sunflower-cake. Among the fodders/top feeds, the mean OM digestibility of FMS was significantly ($P<0.01$) increased by supplementation with lucerne followed by subabul, maize fodder, sorghum fodder and para grass (Fig.1). The IVOMD of FMS was not affected by supplementation with wheat bran, however, supplementation with safflower extraction significantly ($P<0.01$) reduced the IVOMD of FMS. The possible reason may be the less availability of amino acids and peptides which at times limit the efficiency of microbial growth and thereby the digestibility of feed (Preston and Leng 1987).

SUMMARY

The optimum level and source of locally available concentrate ingredients and green fodders/top feeds were determined to improve the *in vitro* digestibility of fingers millet straw (FMS).

The *in vitro* OM digestibility of FMS could be increased by supplementation with maize gluten meal, groundnut-cake, copra-cake, cottonseed extractions, deoiled rice bran, sunflower extractions, lucerne, subabul, maize fodder and sorghum fodders.

REFERENCES

AOAC.1990. *Official Methods of Analysis*. 15th edn. Association of Official Analytical Chemists, Washington, DC.

ARC. 1980. *Nutrient Requirements of Ruminant Livestock*. Agriculture Research Council, Commonwealth Agricultural Bureau, Farnham, Royal, UK.

Preston T R and Leng R A. 1987. *Matching Ruminant Production Systems with Available Resources in the Tropics and Subtropics*. 83-92. Penambul books P.O. Box 512: Armidale, Australia.

Sampath K T, Chandrasekharaiah M, Anandan S and Thulasi A. 1999. Bypass protein for ruminants, *Technical Bulletin* No.2, National Institute of Animal Nutrition and Physiology Bangalore, India.

Snedecor G W and Cochran W G. 1968. *Statistical Methods*. 6th edn. Oxford and IBH. Publishing House, Calcutta, India.

Van Soest P J, Wine R H and Moore L A. 1966. Estimation of true digestibility of forages by the *in vitro* digestion of cell walls- *Proceedings of International Grassland Congress* 438-41.

Indian Journal of Animal Sciences 73 (10): 1187-1188, October 2003

Incidence of abnormal calvings and their effect on milk production of Murrah buffaloes

S TARAPHDER[1], S S TOMAR[2] and A K GUPTA[3]

National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 16 July 2002; Accepted: 4 June 2003

Key words: Abortion, Dystocia, Milk yield, Premature birth, Still-birth

Buffaloes, chief milk producers of our country, play a very important role in dairy economy. Abnormal calving is one of the major factors reducing the milk and affecting the production potential of recently calved buffaloes. Abnormal calving affects the genetic gain of dairy herd, and is responsible for eventual removal of cows from the herd.

The present investigation was carried out on Murrah buffaloes maintained at National Dairy Research Institute, Karnal (Haryana). The data on abnormal calvings (dystocia, abortion, still-birth and premature birth) were obtained from 2306 lactation records of 781 Murrah buffaloes for 16 years (1985 to 2000). Four seasons based on prevailing climate conditions were winter (Dec-March), summer (April-June), rainy (July-Sep) and autumn (Oct-Nov). The incidence of various abnormal calvings were calculated parity- period- and season-wise.

The overall incidence of abnormal calving was 2.66% among 2306 total calvings (Table 1). On an average, the calving difficulty (dystocia) was observed in 1.78% of the cases whereas the abortion, still-birth and premature births were recorded as 6.55, 2.30 and 2.13% respectively. Nearly similar values for abnormal calving have been reported by Basu and Ghai (1980) and Pandit *et al.* (1982) for Murrah buffaloes. On the other hand, lower incidence of overall abnormal calving were reported Bhalaru and Tiwana (1986), Tomar and Verma (1987) and Tomar and Tripathi (1995) for Murrah buffaloes.

Table 1. Incidence (%) of abnormal calving in relation to different non-genetic factors

Effect	No. of observations	Abnormal calving				
		Dystocia	Abortion	Still-birth	Premature birth	Total
Overall	2306	1.78(41)	6.55(149)	2.30(53)	2.13(49)	12.66(292)
Parity						
1	651	3.07 (20)	5.07 (33)	3.38 (22)	1.54 (10)	13.06 (85)
2	503	1.19 (6)	8.94 (45)	1.59 (8)	1.19 (6)	12.92 (65)
3	381	1.05 (4)	6.56 (25)	0.79 (3)	2.36 (9)	10.76 (41)
4	269	1.12 (3)	5.95 (16)	2.60 (7)	2.60 (7)	15.98 (43)
5	189	1.06 (2)	2.65 (5)	2.12 (4)	2.65 (5)	8.46 (16)
≥ 6	313	1.92 (6)	7.98 (25)	2.88 (9)	3.83 (12)	16.61 (52)
Periods						
1985-1988	599	2.17 (13)	5.84 (35)	2.17 (13)	3.00 (18)	13.19 (79)
1989-1992	532	1.13 (6)	4.89 (26)	2.26 (12)	1.69 (9)	9.96 (53)
1993-1996	569	2.11 (12)	6.67 (38)	2.81 (16)	1.76 (10)	13.35 (76)
1997-2000	606	1.65 (10)	8.25 (50)	1.98 (12)	1.98 (12)	13.86 (84)
Seasons						
Winter	629	2.07 (13)	6.83 (43)	1.91 (12)	2.23 (14)	13.04 (82)
Summer	446	1.79 (8)	10.54 (47)	2.92 (13)	1.12 (5)	16.36 (73)
Rainy	823	2.19 (18)	4.86 (40)	2.06 (17)	2.92 (24)	12.03 (99)
Autumn	408	0.49 (2)	4.66 (19)	2.69 (11)	1.47 (6)	9.31 (38)

Figures in parentheses indicate the number of cases.

Present address: [1]Veterinary Surgeon, Khidirpur, Harish chandrapur II, Malda, West Bengal.

[2]Principal Scientist, [3]Senior Scientist, Dairy Cattle Breeding Division.

Table 2. Effect of abnormal calving on milk yield

Effect/ parity	305 days milk yield (mean ± SE) (kg)			Total milk yield (mean ± SE) (kg)		
	Normal calving	Abnormal calving	Difference (%)	Normal calving	Abnormal calving	Difference (%)
Overall	1808.28±81.87 (2039)	1503.13±85.71 (137)	16.87**	1929.50±91.68 (2039)	1547.88±99.03 (137)	19.80
First	1644.57±121.08 (571)	1375.49±122.29 (45)	16.36*	1730.39±269.02 (571)	1465.09±156.44 (45)	15.32**
Second	1936.46±275.09 (442)	1613.95±186.40 (25)	16.68**	2085.33±304.01 (442)	1690.67±214.27 (25)	18.94**
Third	2022.56±242.15 (345)	1710.18±252.67 (20)	15.43**	2107.52±191.85 (345)	1789.67±290.28 (20)	15.09*
Fourth	2199.15±172.59 (238)	1939.61±247.52 (150)	11.82	2390.24±491.85 (238)	2027.36±284.96 (15)	15.19
Fifth	1787.84±315.58 (180)	1527.58±360.52 (9)	14.55**	1797.63±811.41 (180)	1545.04±409.62 (9)	14.02**
Sixth and above	1625.00±281.35 (263)	1422.06±273.07 (23)	12.49**	1653.42±278.61 (263)	1442.89±303.89 (23)	12.76

Figures in parentheses indicate the number of cases; * significant (P<0.05); ** significant (P<0.01).

The incidence of abnormal calving varied from 8.46 to 16.61% among the different parities of Murrah buffaloes. Though the parity had significant (P<0.01) effect on this trait, yet there was no definite trend observed among the parities of Murrah buffaloes. The incidence of dystocia and still-birth was observed higher (3.07 and 3.38% respectively) in heifer calving. Lower and almost similar incidence was observed in rest of the parities. Higher incidence of distocia and still-birth in heifer calving may be due to improve physiological development of growth. So better management is required for reducing their incidence.

Second period had the lowest incidence of abnormal calving (9.96%) and highest was found in the fourth period (13.86%). Period of calving had no significant effect on dystocia, abortion, still-birth and premature birth.

The incidence of abnormal calving was lowest among those calved during autumn (9.31%) than those calved during summer, winter and rainy season (16.36%, 13.04% and 12.03% respectively). The effect of season of calving was significant (P<0.05) on total abnormal calving. Similar results have been reported by Tomar and Verma (1987), Tomar and Tripathi (1995) for Murrah buffaloes. On the other hand, the contrary results have been observed by Kumar and Jain (2000) for Surti buffaloes.

The results further showed that abnormal calving had adverse effect on overall lactation yield as reflected by 17 and 20% less yield up to 305 days and complete lactation duration, respectively, when compared to normol lactation records (Table 2). Reduction in milk yield as measured by difference in the performance of abnormal calvings and normal calvings, varied from 12 to 17% in yield up to 305 days and from 13 to 19% in total lactation yield in different lactations.

SUMMARY

Based on 2306 calving records on 781 Murrah buffaloes maintained at NDRI herd, the overall incidence was 12.66% comprising dystocia (1.78%), abortion (6.55%), still-birth (2.30%) and premature birth (2.13%). The incidence of dystocia was higher in heifer calvings. Analysis showed that the parity as well as season had statistically significant effect on abortion and total abnormal calvings. The heritability and repeatability estimates of abnormal calving traits were low. Abnormal calving had adverse effect on overall 305 days milk yield and complete lactation milk yield when compared to normal lactation records.

REFERENCES

Basu S B and Ghai A S. 1980. Note on age structure, herd life and replacement rate in Murrah buffaloes herd. *Indian Journal of Animal Sciences* **50**: 757-59.

Bhalaru S S and Tiwana M S. 1986. Effect of body condition at calving disorders in buffaloes. *Indian Veterinary Journal* **63**: 851-53.

Kumar H and Jain L S. 2000. Replacement rate in Surti buffaloes. *Indian Journal of Dairy Sciences* **53**: 99-102.

Pandit R K, Gupta S K and Rehman S R P. 1982. Incidence of various reproductive disorders in cattle and buffaloes. *Livestock Advisor* **1**: 51-55.

Tomar S S. 1984. 'Inheritance of certain threshold characters in buffaloes.' Ph.D. Thesis submitted to Kurukshetra University, Kurukshetra (Haryana).

Tomar S S and Tripathi V N. 1995. Genetic and non-genetic variation in abnormal calving of Murrah buffaloes. *Indian Veterinary Journal* **72**: 153-56.

Tomar S S and Verma G S. 1987. Factors affecting parenatal losses in Murrah buffaloes. *Indian Veterinary Journal* **64**: 488-93.

Indian Journal of Animal Sciences **73** (10): 1189-1191, October 2003

Annual hair yield attribute in indigenous camel breeds

CHAMPAK BHAKAT [1], S C MEHTA[2] and M S SAHANI[3]

National Research Centre on Camel, Jorbeer, Bikaner, Rajasthan 334001 India.

Received : 2 August 2002; Accepted : 9 June 2003

Key words: Camel, Dromedary, Hair, Production

In order to obtain optimum profit from camel rearing it is essential to study effective utilization of production of camel hair. The camel hair is being utilized in India since ancient times. It is used in village cottage industry for preparation of common utility items, viz. rope, blankets, floor rugs, bags and mattresses etc. The handicraft articles made up of camel hair, provide work to rural women in the field of grading of hair, tops preparation, spinning of hair, weaving, embroidery with 100% specialty hair and blending with sheep wool, goat hair, cotton and other products. The camel hair is widely used in rural cottage industry of Rajasthan and Gujarat for preparation of various items (Sahani and Khanna 1993). The hair of dromedary camels are durable, strong and have low conductivity (Mukasa 1981). It has been estimated that a camel hair fabric of 620 g weight will be as warm as a pure wool fabric of 900 g weight (Khanna and Rai 1991). The main body sites of hair coverage in the dromedary camels are shoulders, mid portion of body, neck and hump regions. The preliminary results of camel hair blends with wool, silk waste and polyester have shown encouraging results (Gupta *et al.* 1987, 1989). Blended products may also be prepared with sheep wool, goat hair and cotton. Patni and Dhillon (1988) reported that it is worth while to blend camel hair with polyester, wool or silk waste. The camel hair is stronger and warmer as compared to wool (Khanna and Rai 1990). The total world camel population is estimated to be 19.286 million of which India has third highest camel population of 1.52 million (FAO 1998) after Somalia and Sudan. Therefore, analysis of hair production characteristics of various breeds, sex, age group and year of production etc. are of great importance in exploiting the hair production potential and quality of hair for its future utility as pure camel hair as well as blended with other animal fibres, synthetic waste fibres. It is also important in the rural cottage industry. Thus the present investigation was undertaken as an attempt to evaluate the production potential of different breeds and to suggest suitable improvement strategies.

The hair production from 816 camels (*Camelus dromedarious*) belonging to the National Research Centre on Camel, Bikaner, were meticulously recorded and analysed. The data on annual hair yield from different breeds, viz. Bikaneri (359), Jaisalmeri (325) and Kachchhi (132) were recorded. The camel belonged to different age group, viz. 1 year (104), 2 year (93), 3 year (106), 4-6 year (152) and above 6 year (361). Annual hair yield data from different year's like 1998 to 1999 (159), 1999 to 2000 (204) and 2000 to 2001(227) and 2001-2202 (226) were utilized. All the camels were managed under semi- intensive system of management and were daily sent for grazing / browsing for about 6 hr and offered dry fodder (@ 2% of body weight) in the evening time after return from grazing. The hairs were sheared from all over body regions of camel and were clipped by using hand machine during the last week of March of every year. The sheared hair of individual animals were collected in polythene bags after proper skirting (removal of dust and other vegetable matters). The individual camel hair weighted in 5 kg capacity balance with minimum division of 10 g. The recorded data were classified according to various breeds, sex, age and year of production etc. The recorded and classified data were subjected to analysis by applying mixed mode least-squares and maximum likelyhood computer programme (Harvey 1987).

Least-square means along with standard error for annual hair production (kg) of Indian camel breeds are presented in Table 1. The Bikaneri breed of camel produced maximum annual hair yield, followed by Jaisalmeri and Kachchhi breed. The mean production in Bikaneri, Jaisalmeri and Kachchhi were 0.967 ± 0.017 kg, 0.733 ± 0.016 kg and 0.624 ± 0.018 kg respectively. Similar trend of observation was also reported by Sahani *et al.* (1996, 1998). Camel of Bikaneri breed produces more quantity of hair probably due to greater body size and the staple length was also higher in this breed as compared to other 2 breeds. The higher staple length is one of the main causes which leads to higher annual hair production in Bikaneri breed. Yadav *et al.* (2000) reported that young Bikaneri camels indicated higher staple length as compared to young Jaisalmeri and Kachchhi camel. The overall annual hair production was 0.778 ± 0.011 kg. The

Present address: [1] [2] Scientist, [3] Director.

Table 1. Least-square mean ± SE for annual hair yield (kg) of Indian camel breed

	Bikaneri			Jaisalmeri			Kachchhi			
Age (year)	M	F	P	M	F	P	M	F	P	Overall
1	0.804	0.711	0.758	0.639	0.575	0.607	0.634	0.505	0.569	0.630**
	± 0.061	± 0.057	± 0.043	± 0.048	± 0.056	± 0.037	± 0.069	± 0.063	± 0.047	± 0.026
	(20)	(23)	(43)	(25)	(18)	(43)	(8)	(10)	(18)	(104)
2	1.318	1.003	1.161	0.884	0.754	0.819	0.674	0.644	0.659	0.902 * *
	± 0.063	± 0.065	± 0.045	± 0.049	± 0.059	± 0.039	± 0.069	± 0.066	± 0.048	± 0.027
	(19)	(18)	(37)	(23)	(16)	(39)	(8)	(9)	(17)	(93)
3	1.548	1.238	1.394	1.053	0.905	0.979	0.811	0.714	0.763	1.076 * *
	± 0.063	± 0.055	± 0.042	± 0.048	± 0.063	± 0.039	± 0.059	± 0.055	± 0.040	± 0.025
	(19)	(25)	(44)	(24)	(14)	(38)	(11)	(13)	(24)	(106)
4 - 6	0.896	0.816	0.856	0.765	0.703	0.734	0.675	0.581	0.628	0.735 * *
	± 0.056	± 0.041	± 0.035	± 0.044	± 0.046	± 0.032	± 0.062	± 0.046	± 0.039	± 0.021
	(24)	(45)	(69)	(29)	(26)	(55)	(10)	(18)	(28)	(152)
> 6	0.718	0.616	0.667	0.567	0.484	0.525	0.549	0.462	0.506	0.547 * *
	± 0.043	± 0.025	± 0.025	± 0.036	± 0.023	± 0.217	± 0.055	± 0.035	± 0.032	±0.015
	(41)	(125)	(166)	(42)	(108)	(150)	(13)	(32)	(45)	(361)
Overall	1.057* *	0.877* *	0.967* *	0.781* *	0.684* *	0.733* *	0.669* *	0.581* *	0.624 * *	0.778
	±0.026	± 0.022	± 0.017	± 0.020	± 0.023	± 0.016	± 0.028	± 0.024	± 0.018	± 0.011
	(123)	(236)	(359)	(143)	(182)	(325)	(50)	(82)	(132)	(816)

* * - Significant at 1% level. (M – male, F – female, P – pooled).

male Bikaneri camels (1.057 ± 0.026 kg) produced higher hair yield than female (0.877 ± 0.022 kg). Similar trend of observation was found in all other breeds. In all age group cases male camel produces heavier yield than female.

The 3 year (1.076 ± 0.025 kg) age group produced higher quantity of hair, followed by 2 years (0.902 ± 0.027 kg), 4 to 6 (0.735 ± 0.021 kg) and 1 year (0.630 ± 0.026 kg) age group. Above 6 year age group produced lowest quantity of hair yield i.e. 0.547 ± 0.015 kg. The present results are consistent with the observation reported by Bhakat *et al.* (2001). The main reason behind this appears to be the protection provided by nature to the young animals. Table 2 reveals the year-wise least-squares mean along with standard error for annual hair production (kg) in indigenous camels. In all over the 4 year Bikaneri breed (0.939 ± 0.015 kg) produced highest yield. On the contrary the Kachchhi breed (0.648 ± 0.023 kg) produced lowest yield. There was not much variation in annual hair production in different year. Almost similar trend of production was observed all over the years. It ranged from 0.732 ± 0.021 kg to 0.810 ± 0.019 kg. The ANOVA to study the effect of breed, sex, age, year and interaction between genetic group and sex on annual hair production was carried out. The breed significantly ($P< 0.01$) affected the annual hair production. Bikaneri breed of camel significantly ($P< 0.01$) produced higher annual hair yield as compared to the other 2 genetic groups. As expected the sex of camel had a significant ($P < 0.01$) influence on annual hair yield. The male camels of all breeds produced significantly higher annual hair yield as compared to the female camels. Age of camel also significantly ($P < 0.01$) affected the annual hair yield. On the other hand year of production did not affect the annual hair yields significantly. The interaction between genetic group and sex was also found to have significant ($P < 0.05$) effect. The skin coat of younger camels (1 to 3 years age) was

Table 2. Year-wise least-square mean ± SE for hair production (kg) in indigenous camel

Breed	1998 – 1999	1999 – 2000	2000 – 2001	2001 – 2002	Overall
Bikaneri	0.976±0.031	0.898±0.027	0.929±0.026	0.952±0.027	0.939 **±0.015
	(70)	(94)	(101)	(94)	(359)
Jaisalmeri	0.727±0.034	0.716±0.029	0.769±0.028	0.769±0.026	0.746 **±0.015
	(58)	(80)	(89)	(98)	(325)
Kachchhi	0.587±0.046	0.581±0.047	0.717±0.042	0.707±0.044	0.648 **±0.023
	(31)	(30)	(37)	(34)	(132)
Overall	0.763±0.022	0.732±0.021	0.805±0.019	0.810±0.019	0.778±0.011
	(159)	(204)	(227)	(226)	(816)

**Significant at 1% level.

observed to be more soft and finer as compared to adult age group. The Indian dromedary camel breeds showed a wide colour variation ranging from light brown in Jaisalmeri breed to brown, dark brown and blackish colour in Bikaneri and Kachchhi breeds of camel.

The study can be concluded that male camels of Bikaneri breed of 3 years of age produces higher annual hair yield as compared to other age groups, other sex and breeds. It shows enough variation in the annual hair production and hence there exists scope for future improvement in production. The potentiality of camel hair production indicated scope for preparation of blends of camel hair with other natural and synthetic fibres in addition to its traditional use under village conditions. For improving the hair productivity efforts should be made to select superior males within Bikaneri breed of dromedary camel.

SUMMARY

The camel hair and its products can be an important source of additional income for camel keepers. Yearly hair production data from 816 Indian dromedary camels belonging to 3 elite breeds that belong to 5 different age groups were recorded for 4 different years. The mean annual hair production varied form 0.547 ± 0.015 kg (above 6 year age group) to 1.076 ± 0.025 kg (3 year age group). The Bikaneri breed of camel produced maximum annual hair yield followed by Jaisalmeri and Kachchhi breed. The least square analysis indicated significant ($P < 0.01$) effect of breed factors on annual hair production. The male camel produced significantly ($P < 0.01$) heavier annual hair clip than female in all breeds and all age group cases. Annual hair yield was significantly ($P < 0.01$) influenced by sex factor. The highest annual hair production was observed in 3 year age group followed by 2, 4-6, 1 year and 6 years age group. Age of camel had a significant ($P < 0.01$) effect on annual hair production. The interaction between genetic group and sex was significant ($P < 0.05$) on annual hair production.

REFERENCES

Bhakat C, Mehta S C and Sahani M S. 2001. Studies on hair production attribute in Indian dromedary camel managed in an organised farm. *Indian Journal of Animal Sciences* **72** (3): 275-76.

FAO 1998. *Production Yearbook* Rome, Italy.

Gupta N P, Pokharna A K, Arora R K and Sugumar S. 1987. *Mechanical Processing of Wool, Specialty Hair and their Blends.* Annual Report 1987, CSWRI, Avikanagar.

Gupta N P, Patni P C and Sugumar S. 1989. *Properties and Processing of Camel Hair in India.* Indian Textile Journal **99** (4): 180.

Harvey W. 1987. Mixed Model Least-squares and Maximum Liklyhood Computer Programme. United States, Department of Agriculture, New York.

Khanna N D and Rai A K 1990. Scientific camel management can check desertifications. *Indian Farming* **40** (9): 33-35.

Khanna N D and Rai A K. 1991. Camel rearing in the Indian arid zone. *Annals of Arid Zone* **30** (1): 1-10.

Mukasa E. 1981. A Bibliographical Review. *International Livestock Centre for Africa,* P O box 5689, Addis Ababa, Ethiopia.

Patni P C and Dhillon R S.1988. *Areas and Prospects of Utilization of Camel Hair and Hide.* National Seminar on Perceptions and Potentials of Camel Research in Indian, 9-10 October 1988, NRCC, Bikaner.

Sahani M S and Khanna N D.1993. The camel fibre and its prospective utility. *National Seminar on Production and Utilization of Animal Fibres*, Bikaner, 93, December, 13-14.

Sahani M S, Sharma N and Khanna N D.1996. Hair production in Indian camels (*Camelous dromedarious*) managed under farm condition. *Indian Veterinary Journal* **73** (5): 531-33.

Sahani M S, Rathinasabapathy M, Rajendra K and Khanna N D.1998. Factors affecting annual hair production in indigenous breeds of camels (*Camelous dromedarious*) under farm condition. *Indian Journal of Animal Sciences* **68** (3): 267-68.

Yadav B, Mishra B P, Bhakat Champak and Sahani M S. 2000. Hair quality attributes of *Camelus dromedarious. Indian Journal of Animal Sciences* **70** (2): 211-12.

Indian Journal of Animal Sciences 73 (10): 1192-1193, October 2003

Variations of micronuclei in peripheral blood cells of *Channa punctatus*

B KUSHWAHA[1], N S NAGPURE[2], SATISH K SRIVASTAVA[3], RAVINDRA KUMAR[4] and M S VERMA[5]

National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh 226 002 India

Received : 12 July 2002; Accepted : 16 June 2003

ABSTRACT

Genotoxicity was assessed in *Channa punctatus*, collected during different seasons by using micronuclei test. The results showed that there was seasonal variation in frequency of micronuclei in *C. punctatus*. It was 0.15±0.06% (maximum) during spring season and nil in monsoon season.

Key words: Aquatic pollution, *Channa punctatus*, Fish, Genotoxicity

Aquatic environments are often found loaded with large number of genotoxic agents in the form of agricultural pesticides and industrial effluents. As aquatic animals, fishes are most susceptible to genotoxic effect caused by the pollutants. Besides adverse effect on fish germplasm resources, these pollutants may indirectly risk human health through fish consumption. Aquatic pollutants induce formation of micronuclei in peripheral blood cells by condensation of chromosomal fragments or whole chromosomes that are not included in the main nucleus following anaphase stage of cell division (Schemid 1976). Fishes commonly serve as model species for monitoring aquatic pollution. Several studies on assessment of genotoxicity in fishes have gained momentum in recent past using micronucleus test (MNT), sister chromatid exchange (SCE) and comel assay. MNT is popularly utilized by many workers as rapid and dependable indicator of genotoxicity in fishes (Manna *et al.* 1985, Peongothai *et al.* 1996, Castano *et al.* 1998 and Campana *et al.* 1999).

Earlier studies on experimental induction of micronuclei in fishes focused an different aquatic pollutants like agricultural pesticides, chemicals and heavy metals in fishes (Barat *et al.* 1997; Castano *et al.* 1998, Campana *et al.* 1999, Kushwaha *et al.* 2001, Sadhukhan and Manna 1989) have shown MNT to be useful indicator of genotoxicity in fishes. However, there is paucity of information on MNT in fishes from natural habitats, especially on the influence of some factors like season, water availability in ponds etc., on occurrence of micronuclei in fishes. Such information would be valuable in determining level of aquatic pollution and for planning remedial measures to protect fish germplasm from further deterioration and depletion. The present investigation was undertaken to study occurrence of micronuclei in peripheral blood cells of *C. punctatus* collected from natural habitat during different seasons.

Present address: [1-5]NBFGR, Canal Ring Road, Telibagh, P.O. Dilkusha, Lucknow 226 002.

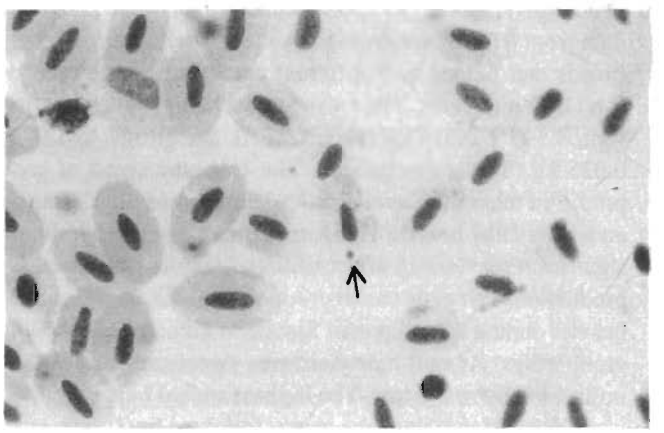


Fig. 1. Blood cells of *Channa punctatus* showing micronucleus.

MATERIALS AND METHODS

Specimens of *Channa punctatus* were collected locally during July 2000, September 2000, December 2000 and March 2001, corresponding to monsoon, autumn, winter, and spring respectively. In each season blood samples were collected individually from caudal veins of 5 specimens on the same day. Blood smears were prepared on clean glass slides and fixed in methanol for 5 min. Slides were then stained with Giemsa, and screened under fluorescent microscope using 100 × objectives for the presence of micronuclei. Micro nucleated red blood cells (RBC) were scored among 1200 to 2200 RBCs/ specimen and frequency of micronucleus (%) was calculated. Frequency % (mean ± SD) of micronuclei observed during 4

seasons was compared by independent samples t-test assuming equal variance (Pagano and Gauvreau 2000).

RESULTS AND DISCUSSION

In specimens collected in monsoon season, micronucleus has not been observed in peripheral blood cells (Table 1). However, micronuclei were observed in post-monsoon collection and the frequency ranged from 0.01 to 0.15. In specimens collected during autumn the frequency of micronuclei was 0.01±0.03, which was statistically insignificant. The frequency of micronuclei observed in December (winter) was 0.09±0.03, which was significantly higher ($P<0.05$) than those in July and September. The maximum frequency of micronuclei (0.15±0.06%) was observed during March (spring) collection, which was significantly different ($P<0.05$) from other 3 collections.

The variation in frequency of micronuclei from monsoon to spring season might be due to variation of pollutant concentration in fishponds attributable to availability of water. During monsoon season due to influx of rain water and inundation of ponds usually result in lower concentration of pollutants. This is reflected by absence of micronuclei in *C.punctatus* as observed in the present study. During post-monsoon, water level in ponds gradually recedes resulting in proportionate increase in pollutant concentrations and hence the higher micronuclei frequency in *C. punctatus* collected during post monsoon and the highest frequency in spring season. Limited informations are available on studies on genotoxicity in fishes from natural habitats. However, Peongothai *et al.* (1996) studied MNT in 5 different fish species of polluted sewage water and reported higher frequencies of micronuclei. The results of the present investigation suggested that genotoxicity is being induced even in fishes from natural habitats, which might be due to presence of some pollutants and its intensity depends on availability of water. Further studies are needed to confirm our finding using advanced techniques like single cell gel electrophoresis so that the data can be used to take remedial measures against depletion of fish germplasm.

Table 1. Frequency of micronucleus in *Channa punctatus*

Month	Blood cells/specimen (mean ±SD)	Micronucleus% (mean ±SD)
July	1293.60±90.93	0.00[a]
September	1317.60±245.52	0.01 ±0.03[a]
December	1172.67±214.51	0.09±0.03[b]
March	2296.00±370.97	0.15±0.06[c]

Values with different superscripts differ significantly ($P<0.05$).

ACKNOWLEDGEMENT

The authors are grateful to the Dr D Kapoor, Director, N B F G R, Lucknow for critically reviewing the manuscript and providing necessary facilities for the present investigation.

REFERENCES

Barat A, Sahoo P K, Nagpure N S and Ponniah A G. 1997. Micronuclei test of peripheral blood in air-breathing fish, *Channa punctatus* treated with Malathion. *Proceedings of the National Symposium on Fish Genetics and Biodiversity Conservation for Sustainable Production* (Eds) Ponniah A G, Das P and Verma S R. Nature Conservators Publication. **05**: 415-17.

Campana M A, Panzeri A M, MorenoV O and Dulout F M. 1999. Genotoxic evaluation of the pyrethraid lambda- cyhalothrin using the micronucleus test in erythrocytes of the fish *Cheirodon interruptus interruptus*. Genetic Toxicology. *Environmental Mutagens*. **438** (2): 155-61.

Castano A, Carbonell G, Carbalb M, Fernandez C, Boleas S and Tarazona J V. 1998. *Ecotoxicol. Environmental Safely*. **41** (1): 29-35.

Kushwaha B, Srivastava S K, Singh B, Nagpure N S and Ponniah A G. 2000. Evaluation of comet assay and micronuclei test as genotoxic assays in *Channa punctatus*. *National Academy of Sciences Letters* **23** (11-12): 177-79.

Manna G K, Banerjee G and Gupta S M. 1985. Micronuclei test in the peripheral erythrocytes of the exotic fish *Oreochromis mossambicus*. *The Nucleus* **28**: 176-179.

Pagano M and Gauvreau K. 2000. *Principles of Biostatistics*. 2nd edn, pp.259-84.Duxbury, USA.

Peongothai K, Shayin S and Vsharani M V. 1996. Induction of micronuclei in fish by polluted water and heavy metals. *Cytobios* **85** (344), 17-22.

Sadhukhan A and Manna G K. 1989. Micronucleus test (MNT) of peripheral blood, gill and kidney cells of the exotic fish *Oreochromis mossambicus* treated with the insecticide ROGOR 30E. *Perspective in Cytology and Genetics* **6**: 331-34 (Eds) G K Manna and U Sinha.

Schmid W. 1976. Micronucleus test for cytogenetic analysis in: (Ed) A Hollaender *Chemical Mutagens, Principals and Methods for their Detection*, Vol. **4**, pp.31-53.Plenum, New York.

International Institute of Agroinformatics & Agromanagement

64 / 28 Arya Nagar, Suraj Kund Road, Meerut - 250001, U.P. (INDIA)
Tel.: 91 - 0121 - 2645294 **Mobile: 9818473366**

E-mail:info@agroinformatics.org Visit us at: www.agroinformatics.org

ADMISSION NOTICE

About the Institute

International Institute of Agroinformatics and Agromanagement has the mission of creating information technology and managerial super house which will work for the agricultural sector. It will result into maximum utilization of available agricultural land and other resources for production purposes and generating agricultural products in abundance.

The Institute aims at career enhancement of its students fostered through exposure to course work, assignments, exams followed by placement assistance through the Institute's placement cell.

Institute Programs

DISTANCE LEARNING
Examination Centres all over India

- **Post Graduate Diploma in Agroinformatics**
- **Post Graduate Diploma in Agromanagement**
- **Diploma in Organic Farming**

Last date to join the current batch is **30th November 2003**

Fee Structure

	Eligibility	Duration	Fee	
Programs			Indian Students	Overseas Students
PG Diploma	Graduation *	1 year	Rs. 5000/-	USD 250
Diploma	10+2	6 months	Rs. 3000/-	USD 200

(*Students pursuing graduation courses in agriculture and allied areas are eligible)

How to Join

The demand for prospectus should be send to the above mentioned address along with a Demand Draft / Personal Cheque of Rs. 110/- or USD 25 in favour of "International Institute of Agroinformatics and Agromanagement" payable at Meerut. Downloaded forms from the website www.agroinformatics.org are acceptable.

Employment Potential

Employment opportunities are available in Private, Public, Joint and Government sectors as Agricultural advisors, Food production managers, Livestock business consultants, Bank managers (agriculture specialist), Agricultural journalists, Farm managers, Farm enterprise managers, College lecturers, Technical sales representatives, Agricultural researchers, Management consultants, Public relations officers, Training providers, Estate managers, Rural development advisors, Quality assurance advisors, Production and technical sales managers, Fertilizer industry, Seed industry, Pesticide industry, Regulatory affairs personnel, Cooperative extension service personnel, forest service officers, crop consultant, Horticulturists etc.

Note

Course Material provided by the Institute is in English only. However, the students have the choice to appear in final examinations either in English or in Hindi.

THE INDIAN JOURNAL OF ANIMAL SCIENCES

GUIDELINES TO AUTHORS

1. *The Indian Journal of Animal Sciences* is published every month. The following types of material will be considered for publication in the Journal.

2.1 PAPERS ON ORIGINAL RESEARCH COMPLETED, not exceeding 3 500 words (approximately 10-12 typed pages), should be exclusive for the journal. They should present a connected picture of the investigation and should not be into parts.

2.2 SHORT RESEARCH NOTES not more than 1 500 words (about 4-5 typed pages). Which deal with (a) resarch result which are complete but do not warrant comprehensive treatment, and (b) descriptions of new material or improved techniques of equipment, with supporting data. Such notes require no headed sections.

2.3 CRITICAL RESEARCH REVIEWS pointing out lacunae in research and suggesting possible lines of future work.

2.4 Contributors are requested to ensure that the research papers or notes submitted for publications have a direct bearing on animal production or open up new grounds for productive research. Basic types of papers and notes which relate to investigations in a narrow specilalized branch of a discipline, may not form an appropriate material for this journal.

3.1 THE TITLE should be short, specific and informative. It should be phrased to indentify the content of the article and include the nature of the study and the technical approach, which is essential for key-word indexing and information retrieval.

3.2 In addition, a SHORT TITLE not exceeding 50 letters should be provided separately for running head lines.

3.3 The BYLINE should contain, in addition to the names and initials of the authors, the place where research was conducted. Present address should be given as a footnote.

4. The ABSTRACT, Written in complete sentences, should not have more than 150 words. It should contain a very brief account of the materials, methods, results, discussion and conclusions, so that the reader need not refer to the article except for details. It should not have references to literature, illustrations and tables.

5.1 The INTRODUCTORY part should be brief and limited to the statement of the problem or the aim of the experiment. The review of literature should be pertinent to problem.

5.2 Relevant details should be given of the animal, MATERIALS AND METHODS, including experimental design and the techniques employed. Where the methods are well known, the citation of a standard work is sufficient. Mean result with the relevant standard errors should be presented rather than detailed data. The statistical methods used should be clearly stated.

5.3 The RESULTS AND DISCUSSION should preferably be combined to avoid repetition.

5.4 The result should be supported by brief but adequate tables, or graphic or pictorial material, wherever necessary. Self-explanatory tables should be typed on separate sheets and carry appropriate titles. The tabular matter should not exceed 20% of the text.

5.5 The data should be so arranged that the tables would fit in the normal lay-out of the page. All weights and measurements should be in metric units.

5.6 The DISCUSSION should relate to the limitations or advantage of the author's experiments in comparison with the work of others.

5.7 Line-drawings should be clearly drawn in black waterproof ink on smooth, tough paper. Photographs should be large, unmounted, glossy prints of good quality. They should be clear and relevant to the subject. Line-drawings and photographs should have legends, which should also be supplied on a separate sheet. Tables and illustrations should not reproduce the same data.

6. A recent issue of the journal should be consulted for the methods of citation of References in the text as well as at the end of the article. The bibliography should have the names of the authors, initials, year of publication, full title of the paper, name of the journal (spelt out in full), volume, preferably the issue within parentheses and complete page-range (not merely the first page). Authors should ensure that all references in the text appear at the end of the paper and vice versa, and that names and dates at the two places correspond.

7. All articles are sent to referees for SCRUTINY and authors should meet criticism by improving the article.

8. Papers should be TYPE-WRITTEN, and double-spaced throughout (including references and tables) on white, durable bond paper of size 22cm × 28 cm, with a 5-cm margin at the top, bottom and left-bottom and left-hand side. Article (including illustrations) should be sent in duplicate, after a careful check-up of typographical errors.

9. Though no rigid rules are recommended for writing authors are requested to consult *The style Manual for Biological Journals*. 4th edn, American Institute of Biological Science, Washington DC.

10. PROOF-CORRECTION should be in ink, in the margin. All queries marked in the article should be answered. Proofs are supplied for a check-up of the correctness of type-setting and facts. Excessive alteration may by charged to the authors. The proofs should be returned within 10 days.

11. Each contributor will receive one copy of the Journal free.

R.N. 14357/57 Delhi Postal Regd. No. DL-18027/2003

Highlights of Forthcoming Issue (November 2003)

• Isolation of *Escherichia coli* of serogroups indicating probable presence of verocytotoxic *E. coli* (*VTEC*) in diarrhoeal kids • Prevalence of *Haemonchus* infection in goats under semi-intensive system • Effect of different electric impulses on development of *in-vitro* matured goat oocytes reconstructed with fetal fibroblast cell • Extraction, purification and characterization of equine chorionic gonadotropin (eCG) from pregnant mare's serum • Typing of food and environmental isolates of *Listeria monocytogenes* by multilocus enzyme electrophoresis • Sero-epidemiology of brucellosis in bovines • Behavioural responses associated with formalin injection into the ear of sheep and rabbits • Evaluation of heterologous microsatellite markers for genetic polymorphism and genetic distancing in indigenous buffalo populations • Sources of variation in production traits and phenotypic and genetic correlations among themselves in Gir cattle • Botanical and nutritional evaluation of different fractions of mustard (*Brassica campestris*) and pearl millet (*Pennisetum typhoides*) varieties at maturity • A software program for digestibility trial calculations • Food preference and feeding habit of fish (*Catla catla, Labeo rohita, Cirrhinus mrigala, Cyprinus carpio*) and freshwater prawn (*Macrobachium rosenbergii*) in rice field refuge

THE INDIAN JOURNAL OF ANIMAL SCIENCES

is indexed in the AGRINDEX, published by the FAO; the *Science Citation Index* and the *Current Contents* (Agriculture, Biology and Environmental Sciences), published by the Institute of Scientific Information, Philadelphia. It is also indexed in the abstracting journals of the CAB International as well as all the major abstracting services of the world.

Printed by Indian Council of Agricultural Research, New Delhi, and published by A Chakravarty, Director (DIPA), on behalf of Indian Council of Agricultural Research, New Delhi, and printed at M/s Aravali Printers and Publishers (P) Ltd., W-30, Okhla Industrial Area, Phase-II, New Delhi 110 020; and published at ICAR, Krishi Anusandhan Bhavan, Pusa, New Delhi 110 012.
Editor: Aruna T Kumar (Assistant Editor)